Exercise induced alterations in NK-cell cytotoxicity - methodological issues and future perspectives

Philipp Zimmer[1,2], Alexander Schenk[1], Markus Kieven[1], Michelle Holthaus[1], Jonas Lehmann[1], Lukas Lövenich[1], Wilhelm Bloch[1]

[1] Department of Molecular and Cellular Sport Medicine, German Sport University Cologne, Am Sportpark Müngersdorf 6, 50933 Cologne, Germany
[2] Division of Physical Activity, Prevention and Cancer, National Center for Tumor Diseases (NCT) and German Cancer Research Center (DKFZ), Heidelberg, Germany.

Abstract

With their ability to recognize and eliminate virus-infected and neoplastic cells, natural killer cells (NK-cells) represent an important part of the innate immune system. NK-cells have attracted the attention of exercise scientists for more than thirty years ago. To date, it is widely accepted that NK-cell counts in the peripheral blood are strongly influenced by acute exercise. Additionally, many studies reported effects of both, acute and chronic exercise on NK-cell cytotoxicity. However, these findings are contradictory. The inconsistence in findings may be argued with different exercise paradigms (type, duration, intensity). Moreover, strongly varying methods were used to detect NK-cell cytotoxicity. This review gives an overview of studies, investigating the impact of acute and chronic exercise on NK-cell cytotoxicity in young and old healthy adults, as well as on specific populations, such as cancer patients. Furthermore, different methodological approaches to assess NK-cell cytotoxicity are critically discussed to state on inconsistent study results and to give perspectives for further research in this field.

Key words: exercise, physical activity, NK-cell, NK-cell cytotoxicity, NKCA

Introduction

Natural killer cells (NK-cells) are part of the innate cellular immune system and have the ability to recognize and eliminate tumor- and virus-infected cells as well as parasites and some types of bacteria.

NK-cells belong to the lymphocytes and its phenotype ($CD56^+$, $CD3^-$) is defined by expression of CD56 and lack of CD3 which is a T-cell surface marker. There are two subpopulations of NK-cells. The first subset is referred to as $CD56^{bright}$ NK-cells due to their high-density surface expression of CD56. They display a low cytotoxic capacity and a high secretion rate of cytokines in response to activation. $CD56^{bright}$ NK-cells represent the minority of the NK-cells and occur mainly in secondary lymphoid tissues (SLT). $CD56^{dim}$ NK-cells represent the majority of the NK-cells in blood (about 90%), spleen, and bone marrow. The amount of CD56 is lower on their surface. However, they characterized by a high cytotoxic capacity (10, 14, 70).

After recognizing and binding to the target cells, NK-cells release a diversity of cytokines, such as interferon-gamma (IFN-γ), tumor growth factor-beta (TGF-β) and interleukin-10 (IL-10) (13, 14, 16). Additionally, they secrete cytotoxic agents such as perforin and granzyme B which are released from cytolytic granules by directed exocytosis (28). IFN-γ increases the activity of other NK cells and activates the innate and adaptive immune system by the stimulation of macrophages and enhancing the cytotoxicity of $CD8^+$ T-lymphocytes(61). TGF-β and IL-10 are immune regulators and have the ability to suppress the immune system.

The activation of NK-cell effector function is regulated by the balance of activating (e.g. CD16, KIR2DS, NCRs, NKG2D, NKp30) and inactivating (e.g. certain KIR receptors, KLRG1, NKR-P1, NKG2A) signals of cell surface receptors, recognizing structures of high molecular weight (38).

NK-cells have attracted the attention of exercise scientists more than 30 years ago. Several studies have shown that absolute and relative NK-cell counts in peripheral blood are strongly influenced by acute physical exercise. Increased NK-cell numbers immediately after cessation of exercise have commonly been reported (69). Depending on the exercise regime (type, duration, intensity), a decrease of NK-cell numbers has been described after a delay of at least 15-30 minutes. This decrease can persist more than 24 hours.

More recent studies have revealed that NK-cell subsets differentially respond to exercise stimuli. Evidence suggests that NK-cells are mobilized from the spleen into circulation by epinephrine dependent β-adrenergic signaling (42). As reported by Dimitrov and colleagues, this mobilization primarily affects cytotoxic ($CD56^{dim}$) NK-cells and is driven by a specific expression of the cell surface markers CD11a and CX3CR1 (15). The knowledge about the redistribution of NK-cells after a delay of exercise is still sparse. Exercise-induced muscle-derived IL-6 was proposed to promote NK-cell infiltration in tumor tissue (53).

Since increased physical activity levels improve survival rates in several neoplastic diseases (51) and elevated NK-cell

Corresponding author:
Dr. Dr. Philipp Zimmer
Fon: 0049 (0) 221 4982 5440, Mail: p.zimmer@dshs-koeln.de

numbers in tumor tissue are associated with a better prognosis (18, 19, 58), NK-cells became one promising target to explain the positive effect of exercise on cancer patients` survival. Furthermore, it was hypothesized that an exercise-induced enrichment of NK-cells could be used for an isolation of these cells in view of further immunotherapeutic strategies (e. g. transplantation) (3).

Besides the intermediate exercise-induced alterations in NK-cell counts, many studies have reported acute and chronic functional changes of NK-cells in response to exercise. However, the results of these studies are inconsistent. In this review a distinction was made between acute effects (single bouts of exercise) and chronic effects (interventions) of exercise on NK-cell cytotoxicity (NKCA) in different populations (young healthy adults, older healthy adults, patient populations). Furthermore, the results of these studies will be discussed against the background of different methodological approaches for detecting NKCA and their translational/clinical relevance.

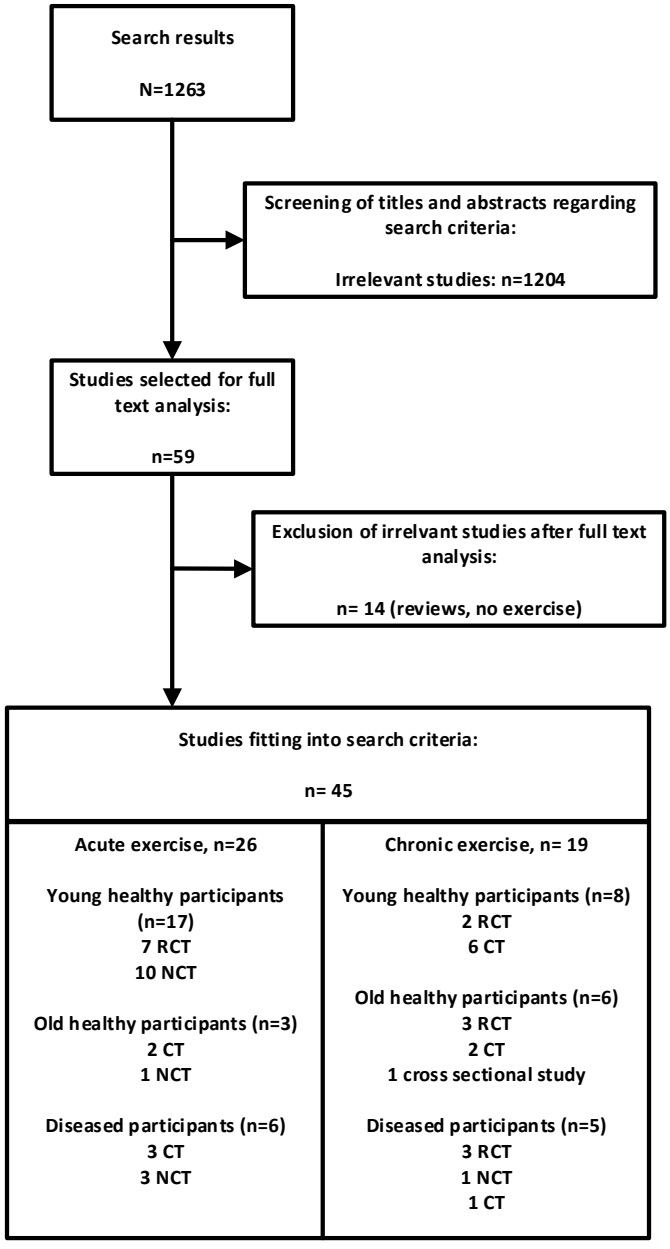

Figure 1: Literature search and results

Methods

A literature search was performed in Pubmed in April 2016. Titles and abstracts were retrieved and screened by three independent reviewers (MK, AS, PZ). The search strategy consisted of a combination of database-specific MeSH terms, free text, and Boolean operators (“AND”, “OR”, “NOT”). The detailed search strategy was performed with the following words: exercise, physical activity, sport, training, natural killer cells, NK-cells, cytotoxicity, cytotoxic, natural killer cell cytotoxic activity, cytolytic activity, NKCA, NK cell function.

Studies were defined as being either randomized controlled trial (RCT), controlled trial (CT), non-controlled trial (NCT), or cross-sectional (observational) study.

Acute exercise was defined as a single bout of exercise followed by assessment of immunological parameters. Chronic exercise was repeatedly performed in the context of an exercise intervention program. Studies on both, acute and chronic exercise employed different types of participants that have been divided into three groups (young and healthy; old and healthy; not healthy). The programs are shortly described by duration, intensity and type of exercise. Moreover, the methods of NKCA determination, the measurements time-points, the outcomes as well as the results are summarized in table 1 and 2.

Results

An overview of literature search and results is given in figure 1.

Acute exercise

Regarding acute exercise 26 studies including 502 participants were selected for analysis. Subjects participated in seven RCTs (157 participants), fourteen NCTs (196 participants) and five CTs (149 participants).

Gannon et al. (20) showed an increased NKCA after moderate cycling at 65% VO_2peak that returned to baseline levels two hours after cessation of cycling. Similar results were reported by other studies using endurance exercise intensities of 50-90% VO_2peak and durations of 20-120 minutes (8, 36, 37, 48, 50, 63, 64). Strasner et al. (64) demonstrated an increasing NKCA in high intensity aerobic exercise (80% VO_2max) compared to moderate exercise (40% VO_2max). These results are in line with those of Nieman et al. (48) who reported a more pronounced increase of NKCA after intensive endurance exercise (80% VO_2max) compared to moderate endurance exercise (50% VO_2max).

Similar to many other studies (37, 40, 43, 50, 52), Shek et al. (63)determined an increasing number of NK-cells immediately after cessation of exercise. While all studies mentioned before used aerobic exercise, Lee et al. (29) applicated Qi-training and found no exercise-induced changes in NK-cell counts. Strasner et al. (64) investigated different intensities and revealed an increase of NK-cell counts after high intensity endurance exercise but not after moderate endurance exercise.

Nieman et al. (48) adjusted the NKCA on a per cell level (NKCApC) and showed a significantly increased NKCApC two hours after recovery of high intensive treadmill running. In contrast Lee et al. (29) showed an increased NKCApC

immediately after Qi-training which returned to the baseline after two hours. Despite these contrary results, other studies found no impact of exercise on NKCApC (20, 37, 50, 64).

Comparing NKCA in old and younger subjects, the results of Woods et al. (75) did not indicate any difference in response to exercise. Ogawa et al. (50) also measured no differences in NKCA and NKCApC but a higher increase of NK-cell counts in elderly untrained subjects after exercise.

Few studies investigated the influence of exercise on patients with specific diseases. Yamanka et al. (77) indicated difference between patients with cervical spinal cord injury (CSCI) and healthy subjects. The patients with CSCI had a constant NKCA during the study in contrast to the able-bodied persons with an increased NKCA immediately after exercise on an arm-crank ergometer. In contrast, Ueta et al. (66) mentioned a decreasing NKCA in patients with spinal cord injury and no difference in NK-cell counts. Furthermore, Furusawa et al. (19) demonstrated a decreasing NKCA after a wheelchair marathon.

Ullum et al. (67) compared HIV+ patients with healthy controls and identified an impaired mobilization of NK-cells and less lysis of target cells in HIV+ patients after exercise. Boas et al. (8) compared NK-cell counts and NKCA in patients with cystic fibrosis and healthy control subjects. After exercise to exhaustion on a bicycle ergometer, NK-cell counts increased in both groups but were significantly higher in the healthy control group. Similar results were reported for NKCA.

Chronic exercise

Regarding chronic exercise 19 studies including 781 participants were selected for analysis. Eight studies were characterized as RCTs (335 participants), nine studies as CTs (380 participants), one as cross sectional study (42 participants) and one as NCT (24 participants).

Moro-Garcia et al. (39) showed increased NK-cell counts and a higher NKCA in athletes compared to non-athletes. In line with these results, Pedersen et al. (51) described higher NKCA in trained subjects compared to sedentary controls. Moreover, Nieman et al. (44) reported elevated NKCA in marathon runners in comparison to sedentary controls, but no differences in NK cell counts. Nieman and colleagues reproduced these results in another study comprising of a 15 week supervised walking program (49). Suzui et al. (65) showed an increase of $CD56^{bright}$ NK-cells during as well as at the end of one month of volleyball training with a decreased NKCA during training. Nevertheless, Roberts et al. (58) found no changes in NK-cell counts, as well as NKCA and NKCApC.

Oppositional to studies including healthy young subjects, most studies with healthy elderly participants did not indicate an influence of exercise on NKCA (11, 46, 55, 56). Nieman et al. (46) revealed an increased NKCA in women with a good physical constitution compared to sedentary controls. However, NKCA of sedentary women did not increase after a twelve week training program. Woods et al. (74) and McFarlin et al. (33) showed an increase of NKCA after a six month aerobic exercise program and a ten week resistance training, respectively. Rincon et al. (57) investigated frail elderly participants and reported an increase in NKCApC after a three month exercise intervention.

Fairy et al. (18) and Peters et al. (54) performed a 15 week and seven month cycling program with breast cancer survivors and found an increase of NKCA after the intervention. Unlike these results Nieman et al. (45) found no influence on NK-cell counts and NKCA after an eight week exercise intervention with moderate weight training and aerobic exercise in a comparable population. Na et al. (41)described an increased NKCA in stomach cancer patients which exercised until 14 days post-surgery. Hagstrom et al. (22) conducted 16 week resistance training with breast cancer patients. The authors did not report any changes of CD107a, a marker for degranulation on NK-cells.

Discussion

Impact of exercise on NKCA

In contrast to the commonly reported blood kinetics of NK-cell counts in response to acute exercise, including an increase immediately after cessation as well as a decrease for up to 48 hours, depending on type, duration and intensity of the exercise session (69), data on NKCA are inconsistent. A tendency could be stated in favor of increased NKCA after more intense aerobic exercise (48, 64). However, such conclusions are restricted by a number of methodological limitations which are discussed in the "methodological issue" section. A potential explanation for the reported increased NKCA immediately after cessation of more intense aerobic exercise could be argued by the epinephrine driven increase in circulating $CD56^{dim}$ (15). Indeed, epinephrine levels have been described to increase with aerobic exercise intensity and persist until 15 minutes after cessation (26). In contrast, epinephrine has also been reported to decrease NKCA in vivo, ex vivo as well as in vitro (35, 59). Additionally, epinephrine is known to primarily mobilize NK-cell subsets with a low expression of the activating receptor NKG2D (3). However, our own research suggests that NKG2D expression increased after prolonged aerobic exercise (79).

Besides epinephrine, other stress related factors such as cortisol and prostaglandins (PGE_2) (12), which are also increased during and after aerobic exercise (27, 31, 62), are associated with a reduced NKCA (35). Against this backdrop, the complex kinetics of catecholamines, prostaglandins and glucocorticoids which differ during and after various exercise modalities should be considered in further studies investigating the influence of acute exercise on NKCA. Finally, it is worth mentioning that NK-cells which are collected from blood during or after acute exercise do not necessarily display the NK-cell proportion which is mobilized and especially migrated in the tissue to eliminate neoplastic or virus infected cells. Although speculative, it might be possible that NKCA of NK-cells which have migrated from the blood stream in different tissues in the following 24 hours after cessation of exercise are influenced by several other (local) factors and are completely independent from the known as "stress hormones". In view of the physical fitness level, studies unanimously revealed elevated NKCA in subjects with a good physical constitution (39, 44, 46, 51, 58). Although Nieman and colleagues (44) showed that physical fitness does not affect NK-cell counts, NKCA might also be influenced by the distribution of NK-cell subsets. More precisely, if physical fit subjects would indicate higher proportions of $CD56^{dim}$ NK-cells, this would result in an increased NKCA(65).

Table 1. Studies on acute exercise and NKCA

Authors	*Year*	*Paper title*	*Subjects*	*n*	*Study design*	*Classification*	*Exercise*	*Period, duration, intensity*	*Methods of NKCA measurement*	*Parameters*	*Time of sampling*	*Results*
Acute exercise –Young healthy participants												
Bigley et al.	2014	Acute exercise preferentially redeploys NK-cells with a highly-differentiated phenotype and augments cytotoxicity against lymphoma and multiple myeloma target	healthy, trained, 30y	16	NCT	no	cycling	3x 30min trials with -5%, +5%, +15% of lactate threshold	PBMC. targets: U266; RPMI-8226; 721.221; 221 AEH; K562. Flow cytometry. NK count / NKCA / NKCA per cell	Cytotoxicity in %	Pre, every 10min, Post, 1h	Highly-differentiated (KIR+/NKG2A) NK cells more redeployed. Shift in proportion of NK. Impact on NKCA against HLA-expressing targets. Post NKCApC↓, 1h NKCApC↑. no effect on K562
Gannon et al.	1998	Beta-endorphin and natural killer cell cytolytic activity during prolonged exercise. is there a connection	male, 26y, recrea-tional active	10	RCT	4x10: Placebo exercise trial / Naltrexone exercise trial, control, non exercise trial	moderate cycling	2h, 65% VO_2peak	PBMC. K562. NK count / NKCA / NKCApC	Cytotoxicity in %	Pre, 1, 2, 4, 24h	NKCA ↑ at t1, t2. NKCA ↓ at t4. NKCApC unaltered
Kappel et al.	1991	Evidence that the effect of physical exercise on NK cell activation is mediated by epinephrine	8 untrained healthy men (20-29y)	2x8 (exercise and epinephrine infusion)	NCT	no	cycling	60 min, 75% VO_2max	BMNC. K562. ^{51}Cr release assay. NKCA	Cytotoxicity in %	Pre, Post, 2h	NKCA ↑ significantly during epinephrine infusion as well as during bicycle exercise / after 2h NKCA ↓ below basal in both / at indentical times no significant differences between NKCA with exercise and epinephrine / Study presents that NKCA induced by physical exercise can be mimicked by the infusion of epinephrine
Kakanis et al.	2010	The open window of susceptibility to infection after acute exercise in healthy young male elite athletes	elite male cyclists	10	NCT	10	cycling	2h at 90% second ventilatory threshold	PBMC. K562. Annexin V. Flow cytometry. NK count / NKCA	Cytotoxicity in %, phenotypes ($CD56^{dim}$ and $CD56^{bright}$)	Pre, Post, 2,4,6,8,24h	NK count ↓ from Pre to 4h&8h. After 24h count = baseline. No signif NKCA and $CD56^{dim}$ change. $CD56^{bright}$ count ↑ only immediately post exercise
Lee et al.	2005	Acute effect of qi-training on natural killer cell subsets and cytotoxic activity	healthy men, 26y	18	RCT	9 + 9 control	Qi-training	1h training	PBMC. LDH release of K562. NK count / NKCApC	Cytotoxicity in %	Pre, Post, 2h after	NKCApC ↑ 60% and returned to baseline within 2h. NK cell count unchanged
McFarlin et al.	2003	Repeated endurance exercise affects leukocyte number but not NK cell activity	young men	10	NCT	4x 10	cycling	3x 20min including 2x 4h recovery	Whole blood. K562 ^{51}Cr release assay. NKCA	Cytotoxicity in %	Pre, Post, 2h, 24h. Pre2, Post2, 2h2, 24h2	NKCA ↑ Post. Returned to baseline after 2h. Greater elevation upon afternoon exercises than upon morning exercises
Miles et al.	2002	The relationship of natural killer cell counts, perforin mRNA and CD2 expression to post-exercise natural killer cell activity in humans	18-40y, moderately trained male runners	10	RCT	6 + 4 control	running	60min,tread mill at 80% VO_2peak	Whole blood. K562 ^{51}Cr release assay. NK count / NKCA / NKCApC	Cytotoxicity in %	Pre, Post, 1,5h, 5h, 24h	NKCApC unchanged. NKCA ↑ by 63% Post; ↓ by 42% at 1,5h in RUN group due to numeric redistribution
Millard et al.	2013	Brief Exercise Increases Peripheral Blood NK Cell Counts without Immediate Functional Changes, but Impairs their Responses to ex vivo Stimulation	25-40y	29	NCT	no	running 50-80 sec	run up+down 150 stair-steps	NK cell isolation: MACS. K562. ^{51}Cr release assay. NK count / NKCApC	Cytotoxicity in %	Pre, Post	NK number ↑. NKCApC not altered

Authors	Year	Paper title	Subjects	n	Study design	Classification	Exercise	Period, duration, intensity	Methods of NKCA measurement	Parameters	Time of sampling	Results
Moyna et al.	1996	Exercise-induced alterations in natural killer cell number and function	healthy male and female 1:1	64	RCT	exercise group, control group	cycling	18min: 3x 6min at 55, 70, 85% VO_2peak	Whole blood ^{51}Cr release assay. K562. NK count / NKCA	Cytotoxicity in %	Pre, 6min, 12min, 18min, 2h	Alterations of NK number (x10) not accompanied by changes of a similar magnitude in NKCA (2x). NKCA ↑. After 2h at baseline
Nieman et al.	1993	Effects of high- vs moderate-intensity exercise on natural killer cell activity	trained men, 17-31y	10	RCT	2x10	moderate treadmill 50% VO_2max vs high intensity 80%	45min	PBMC. K562. ^{51}Cr release assay. NK count / NKCA / NKCApC	Cytotoxicity in %, lytic units	Pre, Post, 1, 2, 3,5h	Moderate: NKCA ↑ Post, below baseline at 1h & 2h. No change of NKCApC. Intense: NKCA ↑ Post, below baseline at 1h & 2h. Significant ↑ of NKCApC from Post to after 2h recovery
Nieman et al.	1995	The acute immune response to exhaustive resistance exercise	male, 47y. 9y weight training	10	NCT	no	parallel leg squat	10 rep at 65% 1-RM every 6sec. 3min rest, new set. until muscular failure. ->9700 +/- 1570kg, 98 +/- 14 rep. 45% VO_2peak	PBMC. K562. ^{51}Cr release assay. NK count / NKCA / NKCApC	Cytotoxicity in %, lytic units	Pre, Post, 2h	NKCA 61% ↓ from Pre to 2h Post. NKCApC ↓ ~40% below Pre level for at least 2h. Suggest prostaglandine from neutrophils and monocytes suppress NKCA. Leg squat exercise to muscular failure results in response of circulating immune cells, like high intense endurance exercise, despite lower % VO_2max and hormonal response
Nieman et al.	2006	Immune changes: 2h of continous vs. intermittent cycling	male trained cyclists, 21y	12	NCT	2x12: continuous cycling vs intermittent cycling	cycling	2 h at 60-65 % $Watt_{max}$. Continuously or with 3min of Rest period every 10 minutes (total time 2h 33 min) 75% VO_2max.	PBMC. K562 labeled with DiO and PI. Flow cytometry. NK count / NKCA	epinephrine, cortisol, interleukins	30 min before exercise, Post and 1h after	No diff in pattern of change between C and R exercise trials. NKCA ↑ Pre to Post. ↓ from Post to 1h below baseline
Pedersen et al.	1988	Modulation of natural killer cell activity in peripheral blood by physical exercise	Healthy, male (23-26y)	6	NCT	no	a) cycling b) back-muscle training	a) 60 min 80% VO_2max b) 5 sets Intervall 10 min = 300 contractions in 1h	BMNC. K562. ^{51}Cr release assay. NK count / NKCA	Cytotoxicity in %	Pre, Post, 2h, 24h	a) NKCA ↑ Pre to Post. Below basal level after 2h. Returned to baseline after 24h b) no significant influence
Shek et al.	1995	Strenuous exercise and immunological changes: a multiple-time-point analysis of leukocyte subsets, CD4-CD8 ratio, immunoglobulin production and NK cell response	male, 22y	6	NCT	no	cycling	2h at 65% VO_2max	PBMC. K562. ^{51}Cr release assay. NK count / NKCA	Cytotoxicity in %	Pre, 30,60,90,120, 150,180,210,240min, 1d, 7d	NK number and NKCA ↑ during exercise. Persistent depression in post-exercise period. 40% ↓ of NK count and NKCA for as long as 7 days. Overtraining -> immunosuppression?

Authors	Year	Paper title	Subjects	n	Study design	Classification	Exercise	Period, duration, intensity	Methods of NKCA measurement	Parameters	Time of sampling	Results
Strasner et al.	1997	Effects of exercise intensity on natural killer cell activity in women	women, 21-33y, oral contraceptives	8	RCT	3x 8 high vs moderate vs control	cycling 80% VO_2max, 40% VO_2max, control	25min per session	PBMC. K562. ^{51}Cr release assay. NK count / NKCA	Cytotoxicity in %	Pre, Post, 90min, 3h	High-Int: NK number ↑and NKCA ↑ post exercise, NKCApC slightly ↑. NKCA ↓ at 90min, NK number like baseline. no diff at 3h. Moderate Intensity: no diff. from control at any time
Wang et al.	2008	Exercise affects platelet-impeded antitumor cytotoxicity of natural killer cell	sedentary men, 22y	37	RCT	ME= moderate exercise SE= servere exercise WUE-SE= severe exercise after warm-up exercise	cycling	ME: 60% VO_2max for 40min SE: up to VO_2max 40min WUE-SE: up to VO_2max + warm-up	target: nasopharyngeal carcinoma cells. Isolation of NK cells (MACS). Flow cytometry. NK count / NKCA	perforin, granzyme B, NK-NPC-binding, caspase activation	Pre, Post	Severe exercise NK count ↑and enhanced NKCA (perforin, granzyme B content) and promotes the platelet-impeded apoptosis induced by NK. Warm-up reduces resistance of platelets increasing NKCA after severe exercise
Wang et al.	2009	Systemic hypoxia affects exercise-mediated antitumor cytotoxicity of natural killer cells	sedentary men, 22y	16	NCT	6x16	cycling	HighE 21%O_2, Mod.E 21%, ME15%, ME12%, breathing in15% and 12% O_2.	nasopharyngeal carcinoma cells. Flow cytometry. NK isolated by MACS. NK count / NKCA	NK-NPC-binding, cellular perforin and granzyme B	Pre, Post, 2h	HE 21%: perforin/granzyme B/IFN in NK, capacity of NK to bind to NPC↑. Breathing at12/15% O_2: no influence. ME 12/15% O_2: NK count, perforin/granzyme B/IFN-g, NK-NPC binding↑
Acute exercise - Old healthy participants												
Bigley et al.	2015	The effect of age and latent cytomegalovirus infection on nk-cell phenotype and exercise responsiveness in man	young ~30y, older ~56y	40	CT	12 CMV+ young, 12 CMV- young, 8 CMV+ old, 8 CMV- old	cycling	30min, 80% VO_2max	PBMC. Flow cytometry. NK count	CD57, CD158, $CD56^{dim/bright}$, KLRG1	Pre, Post, 1h	CMV blunts NK redeployment in young and old. Relatively less $CD57^+$ and $CD158^+$. CMV^{neg} old subjects showed largest NK mobilization. CMV-independent ↑ of $CD57^+$ NK cells during aging. Data suggests: CMV ↓ NK surveillance after exercise in young and old
Ogawa et al.	2005	A single bout of exercise influences natural killer cells in elderly women, especially those who are habitually active	women: trained by walking 64y; untrained 63y; young untrained 25y	24	NCT	8, 8, 8	treadmill walking	a single 30min exercise, 70-75% VO_2peak	BMNC. ^{51}Cr release assay. K562. NK count / NKCA / NKCApC	Cytotoxicity in %	Pre, Post, 2h	↑ of NK count of untrained elderly was higher post-exercise than those of other groups. No difference in NKCA and NKCApC among the three groups. Suggest defect in cytotoxic ability in sedentary elderly; Natural immunity enhanced in daily exercising elderly

Authors	Year	Paper title	Subjects	n	Study design	Classification	Exercise	Period, duration, intensity	Methods of NKCA measurement	Parameters	Time of sampling	Results
Woods et al.	1998	Effects of maximal exercise on natural killer (NK) cell cytotoxicity and responsiveness to interferon-alpha in the young and old	young (18-27y) and old (58-77y), sedentary	47	CT	14 young and 33 old	treadmill, running	2,5 (old) /4mph speed, with 2% increase every 2min until exhaustion.	^{51}Cr release assay. K562 and Daudi cells. PBMC. NK count / NKCA / NKCApC	Cytotoxicity in %	Pre, Post	No diff in NKCA against K562 or Daudi between old and young despite signif higher % of NK cells in old. Maximal exercise -> NKCA↑; Correlation NKCA / NK number in the young, not in the old. Maximal exercise NKCApC ↑against Daudi, not against K562. (IFN to augment NKCA is impaired in the old)
Acute Exercise – Diseased participants												
Bigley et al.	2015	Acute exercise preferentially redeploys NK-cells with a highly-differentiated phenotype and augments cytotoxicity ... Part 2. Impact of latent cytomegalovirus infection and catecholamine sensitivity	(healthy), trained, 30y, with CMV infection	(16) + 6 neue	NCT	no	cycling	LT test; 3x 30min trials: -5%, +5%, +15% LT	PBMC. targets: U266; RPMI-8226; 721.221; 221 AEH; K562. Flow cytometry. NKCA / NKCApC	Cytotoxicity in %	Pre, every 10min, Post, 1h	CMV impairs NK mobilization with exercise when intensity exceeds LT. Latent CMV abated post increase in NKCA. CMV compromises NK cells after acute exercise. Impaired β-AR signaling?
Boas et al.	1999	Immune modulation following aerobic exercise in children with cystic fibrosis	15 subjects with cystic fibrosis and 15 healthy controls	30	CT	patients with CF and healthy controls	cycle ergometer (60 r.p.m.)	max.exercise test to the exhaustion. Power of the ergometer was increased every minute for 10, 15 or 20 W based on the stature of the subjects	PBMC. K562 labeled with PHK-2; PI. Flow cytometry. NK count / NKCA	Cytotoxicity in %	Pre, Post, 60min	Cellular immune response to acute exercise in children with mild or moderate CS appears broadly normal
Furusawa et al.	1998	Short-term attenuation of natural killer cell cytotoxic activity in wheelchair marathoners with paraplegia	spinal cord injuries, wheelchair male marathoners, 27-52y	16	CT	9 + 7 controls with SCI	Wheelchair marathon race		PBMC. ^{51}Cr release assay. T cell leukemia cell line MT2. NK count / NKCA	Cytotoxicity in %	Day before, Post and 1 day after the race	Number of NK cells and NKCA significantly ↓ after the race and returned to Pre-level after 24 h. ↑ post-race adrenaline level, but NKCA ↓. ↓ of NK/NKCA due to overtraining, not due to SCI. Cortisol level ↑ post
Ueta et al.	2008	Attenuation of natural killer cell activity during 2-h exercise in individuals with spinal cord injuries	Subjects with spinal cord injuries (SCI)	13	NCT	7 SCI + 6 able-bodied control	arm ergometer	2h at 60% VO_2max	PBMC. ^{51}Cr release assay, T cell leukemia cell line MT2. NK count / NKCA	Cytotoxicity in %	Pre, 60min, Post	Able-bodied: NKCA ↑ at 60min of exerc, Post and 2h after end of exerc. PGE2 unchanged. SCI: NKCA higher than control at baseline. NKCA ↓ Post, recovered at 2h after exerc. NK cell number lower than in able-bodied and unchanged throughout the experiment. PGE2 ↑ Post, returned to baseline 2h after exerc. Suggested that ↑ of PGE2 in SCI partially contributes to NKCA reduction.

Authors	*Year*	*Paper title*	*Subjects*	*n*	*Study design*	*Classification*	*Exercise*	*Period, duration, intensity*	*Methods of NKCA measurement*	*Parameters*	*Time of sampling*	*Results*
Ullum et al.	1994	The effect of acute exercise on lymphocyte subsets, natural killer cells, proliferative responses, and cytokines in HIV-seropositive persons	8 HIV postiv (26-38yr)	16	CT	8 HIV+ and 8 controls	cycling	60 min, 75% VO_2max	BMNC. ^{51}Cr release assay. K562. NK count / NKCApC	Cytotoxicity in %	Pre, Post, 2h, 4h	Suggestion: HIV+ subjects have impaired mobilization of neutrophils, NK and LAK cells
Yamanaka et al.	2010	Impaired immune response to voluntary arm-crank ergometer exercise in patients with cervical spinal cord injury	persons with CSCI (cervical spinal cord injury) land dysfuncional sympathetic NS, male, chronic injury state	14	NCT	8 patients with CSCI + six able-bodied persons	arm crank ergometer	20 min of exercise with 60 % of VO_2max	PBMC. ^{51}Cr release assay. T cell leukemia cell line MT2. NK count / NKCA	Cytotoxicity in %	Pre, Post, 1h, 2h	Able-Bodied: - NK cell count and NKCA ↑ post-exercise and ↓ 1h later to a lower level than before returning to the baseline after 2 h Conclusion: In subjects with CSCI, lack of NKCA response is probably due to dysfunctional sympathetic NS: no adrenaline response

Table 2. Studies on chronic exercise and NKCA

Authors	*Year*	*Paper title*	*Subjects*	*n*	*Study design*	*Classification*	*Exercise*	*Period, duration, intensity*	*Methods of NKCA measurement*	*Parameters*	*Time of sampling*	*Results*
Chronic Exercise – Young healthy participants												
Moro-Garcia et al.	2014	Frequent participation in high volume exercise throughout life is associated with a more differentiated adaptive immune response	athlets and non-athlets	95	CT	30 young non-ath.; 27 young ath.; 26 elderly non-ath.; 12 elderly ath;	young ath.: rowing, running, resistance training; Old ath.: easy-moderate intesity	young ath: 6d/wk; 2h/d; Old ath: 5d/wk; 80min/d	Whole blood. Flow cytometry. CD107a expression for degranulation. CD69 "NK activation". NK count / NKCA / NKCApC	Cytotoxicity in %	Pre, Post	NKCA ↑; young athletes: NKCA and degranulation significantly increased; young ath. had higher NK counts than old ath; no change in count or structure of NK receptors
Nieman et al.	1990	The effects of moderate exercise training on natural killer cells and acute upper respiratory tract infection	mildy obese women, 25-45y	36	RCT	18 exercise + 18 control	walking	15 weeks; 45min/d brisk walking at 60% max HR; 5d/wk	PBMC. K562. ^{51}Cr release assay. NK count / NKCA	Cytotoxicity in %	Pre, week 6 and 15	NKCA rose strongly after 15 weeks to the same level, in control and exercise group! Fewer upper respiratory tract infection symptoms in exercise group.
Nieman et al.	1995	Immune function in marathon runners versus sedentary controls	marathon runners, ~40y	40	CT	22 + 18 sedentary controls	Conditions: training >4y,>7marathons in less than 3h45min	-	PBMC. Flowcytometry. K562.^{51}Cr release assay. NK count / NKCA / NKCApC	Cytotoxicity in %	one sample	NKCA/NKCApC significantly different: in marathoners elevated. NK cell number similar; suggest chronical elevation; %body fat and VO_2max related antiproportional to NKCA
Pedersen et al.	1989	Natural killer cell activity in peripheral blood of highly trained and untrained persons	male racing cyclists (median 23y), healthy control (median 26y)	42	CT	27 trained + 15 untrained	performance test	none	PBMC. K562.^{51}Cr release assay. NK count / NKCA	Cytotoxicity in %	one sample	NKCA higher in trained persons
Roberts et al.	2004	CD94 expression and natural killer cell activity after acute exercise	highly trained male triathletes, 20-30y	9	RCT	no	Training for competition. test cycling: 20min submaximal exercise, then incremental test until exhaustion	10 weeks; 3x test cycling in lab	PBMC. Flow cytometry. K562.^{51}Cr release assay. NK count / NKCA / NKCApC	Cytotoxicity in %	week 2,5,10: Pre-exercise; 20min after exhaustion	Resting NK numbers and NKCA did not differ over 10 weeks; NK numbers increased post-exercise; Increased NKCA after exercise reflects numbers of NK cells. NKCApC not changed
Suzui et al.	2004	Natural killer cell lytic activity and CD56(dim) and CD56(bright) cell distributions during and after intensive training	female college-level volleyball players + healthy students as control	15	CT	8 + 7 control	heavy pre-season training	1 month: 5h/d; 6d/wk.	PBMC. Flow cytometry. K562.nonradioactive Europium release assay. NK count / NKCA / NKCApC	Cytotoxicity in %	Pre, day 10, one day before end; 1wk after end of training	NKCA ↓ from Pre to End, returned to Pre-level 1wk later. Similar for NKCApC. $CD56^{bright}$NK↑, $CD56^{dim}$ NK number unchanged

Authors	*Year*	*Paper title*	*Subjects*	*n*	*Study design*	*Classification*	*Exercise*	*Period, duration, intensity*	*Methods of NKCA measurement*	*Parameters*	*Time of sampling*	*Results*
Wang et al.	2011	Hypoxic exercise training promotes antitumour cytotoxicity of natural killer cells in young men	sedentary men	60	CT	5 groups with 12	21% O_2 control; 15% O_2 control; 21% O_2 50% max work rate; 15% O_2 50% heart rate; 15%O_2 50% max work rate;	30min/d, 5/wk, 4 weeks.	nasopharyngeal carcinoma cells (NPC). PBMC. NK isolation with MACS-negative immunomagnetic selection. NK count / NKCA	Perforin & granzyme B with flow cytometry; annexin V,propidium iodide staining (FACS) -> % necrotic/apoptotic cells.	48h before and 48h after last training	15% O_2exercices reduce terminally differentiated NK subsets; activating molecules and cytotoxic granule proteins in NK ↑, but no increased anti-NPC-cytotoxicity of NK; $CD56^{dim}$ increased, $CD56^{bright}$ decreased; increase of CD11a and NKG2D; anti-NPC cytoxicity increased
Chronic Exercise - Old healthy participants												
Campbell et al.	2008	Effect of exercise on in vitro immune function: a 12-month randomized, controlled trial among postmenopausal women	postmenop overweight /obese, 50-75y women, healthy, sedentary	115	RCT	53 + 62 control	Moderate aerobic /bicycling exerc. (Beginning: 40% max HR; 60-75% in week 8); C: strechting, relaxing	1 year; Exerc: >45min/d, 5d/wk; C: 1d/wk	PBMC.Flow cytometry. K562 - propidium iodide assay. NK count / NKCA / NKCApC	Cytotoxicity in %	Pre, 3 mo, 12 months.	no effects
McFarlin et al.	2005	Chronic resistance exercise training improves natural killer cell activity in older women	65-86y postmenop women	25	CT	19 + 6 control	resistance training	10 weeks; 3x/wk; 80% 1.RM (first repetition maximum)	Whole blood. K562.^{51}Cr release assay. NK count / NKCA / NKCApC	Cytotoxicity in %	before: Pre, Post, 2h; after 10 wk: Pre, Post, 2h	No significant difference in NKCA but NKCA ↑ in response to an acute bout of exercise
Nieman et al.	1993	Physical activity and immune function in elderly women	sedentery women; 67-85y	30 + 12 + 13	CT	14 walkers (sedentary), 16 control; + 12 highly conditioned; + 13 young healthy not active	walking	30-40min 5d/wk. 12 weeks. 60% heartrate	PBMC. Flow cytometry. K562.^{51}Cr release assay. NK count / NKCA / NKCApC	Cytotoxicity in %	Pre, 5wk, 12wk; (old active at baseline; young inactive at 12wk)	Walkers: no improvement in NK activity after 12 weeks. Highly conditioned at baseline: higher lytic units than walkers despite no diff in NK numbers. Seasonal effects on immune functions
Raso et al.	2007	Effect of resistance training on immunological parameters of healthy elderly women	sedentary women; 60-77y	42	RCT	exercise + control	moderate resistance training	12 mo; 3 sets of 12 repet at 60% 1.-RM for 5 diff exercises; 3x/wk; 60 min/d;	PBMC. Flow cytometry. K562; ^{51}Cr release assay. NK count / NKCA / NKCApC	Cytotoxicity in %	Pre, 6mo, 12mo	No significant difference between groups or according to time for quantitative ($CD56^{dim/bright}$, CD3, ..) and functional immunological (NKCA, ..) parameters
Raso et al.	2012	Immunological parameters in elderly women: correlations with aerobic power, muscle strength and mood state	sedentary elderly women, 60-77y	42	Cross-sectional	-	none	none	PBMC. Flow cytometry. K562.^{51}Cr release assay. NK count / NKCA / NKCApC	Cytotoxicity in %, muscle strength, aerobic power, mood state	one sample	Neither NKCA nor lymphocyte proliferation were correlated with aerobic power or muscle strength; Psychological changes associated with aging may have a substantial adverse effect upon the immune system, and immunological function may be enhanced more by addressing these issues than by focusing upon aerobic or resistance training

Authors	Year	Paper title	Subjects	n	Study design	Classification	Exercise	Period, duration, intensity	Methods of NKCA measurement	Parameters	Time of sampling	Results
Woods et al.	1999	Effects of 6 months of moderate aerobic exercise training on immune function in the elderly	sedentary elderly 65y	29	RCT	14 + 15 control	moderate aerobic exercise	6 months: 3x/wk; at 50% to 65% VO_2max; 10-40min/d;	PBMC. K562. Flow cytometry.^{51}Cr release assay. NK count / NKCA / NKCApC	Cytotoxicity in %	Pre-exercise, post, 20min after exercise	No significant difference in NKCA. Acute exerc response is attenuated in Control and exercise groups post-intervention. NK function was performed only on 7 + 12 subjects
Chronic Exercise – Diseased participants												
Fairey et al.	2005	Randomized controlled trial of exercise and blood immune function in postmenopausal breast cancer survivors	postmenop 50-69y, breast cancer survivor	53	RCT	25 cyclists + 28 control	cycling; 70-75% VO_2max	15wk; 3x/wk; wk1-3: 15min; incremental, wk13-15: 35min;	PBMC. K562.^{51}Cr release assay. NK count / NKCA / NKCApC	Cytotoxicity in %	Pre, week 15.	NKCA ↑
Hagstrom et al.	2016	The effect of resistance training on markers of immune function and inflammation in previously sedentary women recovering from breast cancer: a randomized controlled trial	breast cancer survivor; 18-70y; sedentary	39	RCT	20 + 19 control	resistance training	16 wk; 60min 3x/wk; repetions at 80% 1-RM;	Flow cytometry	markers of NKCA, granzyme B, perforin	Pre; week 17	No change in NK-percentage. No change in granzyme B or perforin. reduced NK cell expression of TNF-α
Na et al.	2000	Exercise therapy effect on natural killer cell cytotoxic activity in stomach cancer patients after curative surgery	stomach cancer patients, 28-75y	35	CT	17 exersice + 18 control	arm + bicycle ergometer	from post-OP day 2: 30 min 2x/d, 5d/wk for 2 weeks. 60% maxHR	PBMC. K562.^{51}Cr release assay. NKCA	Cytotoxicity in %	Post OP days 1, 7, 14	Suggests early moderate exercise has benefincial effect on NK in Sto.Cancer patients after surgery. NKCA in younger and non-metastasis patients more increased
Nieman et al.	1995	Moderate exercise training and natural killer cell cytotoxic activity in breast cancer patients	female breast cancer; undergone surgery, chemo, and/or radiation previously; 35-72y	12	RCT	6 +6 control	moderate weight training and aerobic activity	8 wk; 60min/d; 3d/wk ; 75% HR max;	PBMC.Flow cytometry. K562.^{51}Cr release. NK count / NKCA / NKCApC	Cytotoxicity in %	Pre, Post	NKCA and NK number not significantly altered; Suggests: moderate exercise over 8weeks no significant effects
Peters et al.	1994	Influence of a moderate exercise training on natural killer cytotoxicity and personality traits in cancer patients	breast cancer patients; 49 +/- 6y; stage one or two. >6 months since surgery	24	NCT	no	moderate cycling	7 months; 2-3x week;	NK isolated according to Cosentino and Cathcart. K 562.^{51}Cr release assay. NK count / NKCA / NKCApC	Cytotoxicity in %	Pre; 5 weeks; 7 months;	After 7 months: NKCA of patients in range of healthy people from other studies
Rincon et al.	1996	Exercise in frail elderly men decreases natural killer cell activity	frail male, >70y	13	CT	6 + 7 control	strength, balance, walking, stretching	3months; 60min/d; 3x/wk	Whole blood. Flow cytometry. K562.^{51}Cr release assay. NK count / NKCA / NKCApC	Cytotoxicity in %, lytic units	each Pre + Post: 0wk, 6wk, 12 wk	Exercise increased NKCA transiently Pre/Post; But long-term effect: reduction below basal NKCA; Caution in very frail elderly

In view of chronic exercise interventions, results are contradictory. The heterogeneity in results could also be argued by alterations in "stress hormones". Chronic alteration in baseline levels and differences in the response to acute exercise after training periods in catecholamine-, prostaglandin- and glucocorticoid levels have been reported in several studies with healthy subjects (9, 32, 80). For example, regular exercise is known to reduce resting glucocorticoid- and catecholamine levels. Therefore, decreased levels of these agents which can be found in subjects with a good physical constitution or after a specific exercise intervention could explain an improved NKCA although the proportion of $CD56^{bright}$ may increase (65). When investigating clinical populations, it should be kept in mind that baseline levels and responses to exercise of the named factors are further influenced by several diseases (17, 80). As already mentioned for acute effects of exercise, these factors should be investigated as mediators of alterations in fitness/training-induced NKCA as well.

Methodological issues

Regarding functional NK-cell assessments several approaches have been described. The NK cell function was commonly tested by measuring the NK-cell cytotoxic activity (NKCA), NK cell count and NKCA per cell. Cytotoxic activity assays were frequently performed by mixing either peripheral blood mononuclear cells (PBMC) or isolated NK cells with a target cell line (leukemia cell line K562 in most cases). The percentage of target cell lysis was frequently measured with ^{51}Cr release assay detecting the radioactivity in the samples supernatant (47, 50, 67). However, newer studies utilized non-radioactive agent like Annexin V which was determined by flow cytometry (25). Some research groups had concerns about K562 as a HLA-deficient target cell line. Therefore they used further cell lines with different surface expression patterns like Daudi cells, MT2, U266, RPMI-8226, 721.221, and 221 AEH (4, 75, 77).

Other studies assess the NKCA without counting killed target cells, but by measuring the amount of perforin, granzyme B, IFN- γ, and the NK-target-cell-binding via flow cytometry (73). Further indirect measurements can complete the evaluation of NK cells. The differentiation marker CD57 can be used as target for flow cytometry. CD57 expression is induced on $CD56^{dim}$ NK cells after activation by IL-2. CD57+ $CD56^{dim}$NK-cells are considered to be terminally differentiated and mature. They are characterized by poor cytokine-mediated proliferation, a higher sensitivity to stimulation via CD16 and higher cytotoxicity (30). Moreover, the lysosomal-associated membrane protein-1 (LAMP-1 or CD107a) was reported as marker of NK-cell cytolytic activity. Its surface expression was increased by engaging MHC devoid targets and its expression levels correlated with both, cytokine secretion and lysis of target cells. However, a large NK-cell subset did express CD107a while it did not secrete cytokines. Therefore, it was suggested that CD107a could be used as marker of NK-cell activity and identification of a large degranulation fraction of activated NK-cells (1).

As pointed out in table 1 and table 2 several different approaches have been used to assess NKCA. Against this background, results of studies are hardly comparable. NKCA was frequently measured using PBMCs (4–7, 11, 18–20, 29, 41, 43–49, 51, 55, 56, 58, 63–66, 72, 74, 75, 77) whereas other studies incubate tumor cells with whole blood samples (33, 34, 36, 39, 40, 57, 57). These approaches have some major limitations. First, both methods include other cells than NK-cells with tumor-competitive properties, such as cytotoxic T-cells. Therefore, statements on specific functional changes of NKCA are restricted. Second, the use of whole blood samples comprises various other agents, such as cytokines and hormones which may influence the target cells itself. However, Gotlieb and colleagues suggest that in vitro and ex vivo assays usually lead to an overestimation regarding the reported "stress hormone" induced suppression of NKCA (21). The authors propose that further research should use whole blood sample approaches, arguing that such attempts reflect the in vivo situation more precisely. We absolutely agree with this opinion. Nevertheless, one should keep in mind that incubating tumor cells with whole blood samples does not represent the in vivo situation (tumormicromilieu) as well.

Third, it is worth to mention that NKCA should be quantified on a per cell level. This is relevant since NK-cell numbers can strongly vary between pre- and post-exercise conditions. Just in a few studies NK-cells were isolated (e. g. by magnetic beads) to detect cytotoxicity (25, 37, 54, 71, 73). To minimize NK-cell-specific alterations, a negative selection is strongly recommended.

Furthermore, studies showed that NK-cell subset distribution is influenced by both, acute and chronic exercise (25, 65, 72). Since NK-cell subsets display different cytotoxic potentials, changes in these fractions should also be considered when analyzing NKCA.

Another issue, which might be of clinical relevance, is the type of tumor cells which is used as target for detecting NKCA. Especially in clinical studies with cancer patients, e. g. breast cancer, it would make sense to measure NKCA against a breast cancer cell line, whereas using the leukemia cell line K562 is of inferior interest (with the exception of the genesis of secondary neoplastic burdens). This issue becomes even more important since first studies have shown that NKCA depends on the type of target cells (e. g. nasopharyngeal carcinoma cells (71–73), Daudi (75), U266 (4, 5), RPMI-8226 (4, 5), 721.221 (4, 5), 221AEH (4, 5) or T-cell leukemia cell line MT2 (19, 66, 77)).

Some studies determined NKCA by measuring the amount of perforin, granzyme B, IFN-γ and the NK-target-cell-binding via flow cytometry (73). To get more knowledge about the mechanistic underpinnings, a combination of both direct and indirect methods seems to be a promising strategy for further research. In addition, the expression of activating and inhibiting NK-cell-receptors should be taken into account. Exercise has been described to alter NK-cell receptor expression (79). Therefore, changes in NK-cell target killing might not be reasoned by in- or decreased levels of cytotoxic agents, but by a modification of surface receptor expression.

Finally, acute effects of NKCA can persist longer than 24 hours (63). Therefore, measurement time points in studies investigating chronic effects of exercise should be chosen carefully (measurements up to 24 hours after the last training session might still display acute effects).

Due to heterogeneous methods, strongly varying exercise interventions and measurement time points, we decided that quantitative analysis (meta-analysis) does not make sense so far.

Although the significance of current literature on the influence of exercise on NKCA is restricted and needs further approval, there is evidence that at least some positive effects, such as an improved defense against neoplastic cells is based on NK-cell mobilization and activation (53). In fact, increased NK-cell numbers in tumor tissue are associated with improved prognosis in different cancer species (23, 24, 68). Moreover, exercise is known to have preventive effects regarding cancer risk and to reduce cancer specific mortality (2, 60, 76, 78). Therefore, research on NK-cells in the context of exercise and cancer was and will be a highly relevant topic for further investigations.

Conclusion

In summary, at least some exercise/training modalities seem to impact NKCA. As potential mediators of these effects, the role of catecholamines, prostaglandins as well as glucocorticoids warrants further investigation. On a molecular level, epigenetic alterations might be involved in functional changes of NK-cells. Currently, exercise studies on NKCA are hard to compare since different exercise regimes (type, duration, intensity, and frequency) were used. Varying measurement time points as well as the use of different methods to assess NKCA delimitate the comparability of the studies. Independently of the methods which will be used to detect NKCA in the future, an additional characterization of NK-cell subsets as well as the assessment of potential mediators (e. g. epinephrine, cortisol) is strongly recommended. Further research is needed to clearly identify the impact of exercise on NKCA.

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