The effect of exercise on regulatory T cells: A systematic review of human and animal studies with future perspectives and methodological recommendations

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ABSTRACT

Many of the exercise-related health-promoting effects are attributed to beneficial immunomodulation. The restoration of immune homeostasis is context-dependent, meaning either to increase anti-inflammatory signaling to counteract disease progression of non-communicable (auto)inflammatory diseases or to enhance (local) activity of proinflammatory immune cells to slow down or inhibit cancer progression. Regulatory CD4$^+$ T cells (T_{regs}) represent the main regulatory component of the adaptive immune system that fine-tunes inflammatory responses, keeps them in check and prevents long-lasting autoimmunity. Because often dysregulated in the context of various diseases, emerging treatment approaches aim to modulate their number or inherent anti-inflammatory and immunosuppressive function in a highly disease-specific way. Exercise represents a non-pharmacologic strategy in disease prevention and rehabilitation and may be an effective treatment with few to no side effects to counteract dysregulation of T_{regs}. To date, several studies have evaluated the effect of exercise on T_{reg}-related outcomes. This review aims at providing a comprehensive overview on alterations of blood- or tissue-derived T_{reg} counts, proportion and functionality following acute and chronic exercise in humans and animal models. From the 60 reviewed studies, an overall disease-specific beneficial effect of chronic exercise on T_{reg} levels in animal models can be stated, while both acute and chronic effects in human studies are less definite. However, T_{reg} phenotyping is less sufficient in the animal studies compared to human studies. Only a limited number of studies investigated T_{reg} functionality. There is a large heterogeneity concerning study design, human population or animal model, exercise protocol, and T_{reg} outcome measure specification which makes it difficult to compare results and draw clear conclusions. Study results are discussed in the context of current concepts in exercise immunology. Finally, future perspectives and methodological recommendations are provided to promote research in this field.

Keywords
Exercise, Physical activity, Immune homeostasis, T_{regs}, Exercise immunology

INTRODUCTION

The proper functioning of immune cells with regulatory function is indispensable for a balanced and fine-tuned immune system in mammals. Within the CD4$^+$ T cell compartment, specialised regulatory cells (T_{regs}) exist that are characterized by CD25 upregulation, CD127 downregulation and expression of the transcription factor forkhead box P3 (Foxp3) as the master regulator in T_{reg} development and function. They mature in the thymus as a small subset of positively selected thymocytes that receive signals slightly weaker than those which are negatively selected by clonal deletion (51). Consequently, their T cell receptors possess a high affinity for MHC:self peptide complexes. However, activation of T_{regs} leads to contact-dependent (e.g. via CTLA4-B7 interaction or gap junction formation) and contact-independent (mainly via cytokines) suppression of effector immune cells from the innate and adaptive immune system (12, 83), which favors immune homeostasis and self-tolerance, and prevents excessive inflammation. This becomes apparent in cases of genetic ablation or mutations in the Foxp3 gene which lead to severe disease phenotypes in mice (14) and humans (8). T_{regs} can also be derived from naïve CD4$^+$ T cells in the periphery by the orchestrated action of certain cytokines, however, there is no clear method to distinguish between the thymus-derived and peripherally induced subsets (52, 131). Due to their "immune harmony" role, T_{regs} have been investigated thoroughly in the context of several disease states where immune dysregulation plays a key role in pathogenesis and disease progression (see table 1), but also in transplantation and aging (55, 122). Thus, treatment approaches that target T_{reg} metabolism, and therefore functionality, are a hot topic in autoimmunity, cancer, and organ transplantation (113). Although it is proposed to stain at least for CD4, CD25, and Foxp3 to identify human and animal T_{regs} (112), species-specific differences in both the T_{reg} phenotype and the overall immune system makeup in general need to be considered (81, 86, 114). Other commonly used markers are those for defining the maturation status with CD45RA$^+$CD45RO$^-$CD95$^-$ T_{regs} being naïve and CD45RA$^-$CD45RO$^+$CD95$^{+/high}$ T_{regs} representing a highly differentiated or memory phenotype (93).

Regular physical activity and exercise training are widely accepted determinants of a healthy lifestyle with implications for disease prevention and partial rehabilitation. In contrast to medical treatments, exercise does not cause any side-effects which makes it an attractive supportive therapy option

in many disease conditions. There are several existing concepts addressing why exercise exerts those effects. However, mainly due to complex inter-organ crosstalk, the underlying tissue-specific cellular and molecular mechanisms in response to exercise are unclear, and the focus of ongoing research (95, 145). Accumulating evidence in the field of exercise immunology revealed that the immunomodulatory characteristics of exercise may account for some health-promoting effects (73, 138). The positive impact of exercise-induced immunomodulation is attributable to the long-term establishment of a more anti-inflammatory state on the systemic level since most of the Western diseases arise from a phenotype of chronic silent inflammation as a consequence of metaflammation and inflammaging (50). Other existing concepts refer to senescence and redistribution of immune cells within body compartments. The former concept describes the idea of an exercise-induced decline in senescent or exhausted T cells, e.g. via apoptosis, to "make space" for newly built, naïve T cells, which ultimately results in improved immunocompetence (123). The latter concept describes the (re)entry of lymphocytes from margination pools into the circulation in response to acute exercise, reflected by an increased number of cells, that is followed by the migration of lymphocytes back to tissues that generate strong chemotactic signals.

Most of the research has been conducted in effector $CD8^+$ T cells and Natural Killer cells. However, there is a lack of knowledge on the effects of acute and chronic exercise interventions on the anti-inflammatory arm of $CD4^+$ T cells. Since T_{regs} represent the main and best studied regulatory part within the adaptive immune system, they may play a major role in exercise-induced health promotion by the restoration of local and systemic immune homeostasis. Therefore, this review should inform about all human and animal research that focused on the impact of acute and chronic exercise interventions and the physical activity level on T_{reg} cell numbers, proportions, functionality and T_{reg}-related outcomes (i.e. Foxp3 gene expression). Limitations concerning study design, choice of outcome measures, and heterogeneity between studies are discussed. To promote and improve further research, methodological recommendations and promising research questions in the context of current concepts in exercise immunology are provided.

Table 1: Regulatory T cell characteristics in major disease states.

DISEASE STATE	T_{REG} NUMBER/FUNCTIONALITY	CONSEQUENCE	REF
Autoimmunity	*Decreased/Dysfunctional*	*Increased activity of autoreactive immune cells*	(28)
Cancer	*Increased*	*Decreased activity of cytotoxic immune cells in the tumor microenvironment*	(103)
CNS pathologies	*Decreased/Dysfunctional*	*Increased neuroinflammatory signaling in several brain and spinal cord regions*	(34)
Cardiovascular diseases	*Decreased/Dysfunctional*	*Decreased anti-inflammatory counter-regulation in the vasculature*	(85)

METHOD

This study was conducted in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) (90). Since research on $CD4^+CD25^+$ regulatory T cells started after the publication of the seminal paper of Sakaguchi et al. in 1995 (118), the literature search considered a period of the last 25 years (1995 to April 1st, 2020). To not miss any relevant published study in this field, the references of included studies were screened.

Search strategy

The literature search was conducted using MEDLINE (via PubMed), Web of Science, and SportDiscus for human studies and MEDLINE (via PubMed) and Web of Science for animal studies. Search strategies involved Medical Subject Headings (MeSH) and text words combined through Boolean operators (''AND'', ''OR'').

For human studies, the search string included common synonyms for the concepts of [1] physical exercise, [2] human population, and [3] T_{reg} characteristics: *(exercise OR "physical activity" OR sport* OR training OR HIIT OR HIT OR cycling OR running OR walking OR swimming) AND (human OR men OR women OR athlete* OR runner* OR cyclist*) AND ("regulatory T cells" OR Treg* OR "Forkhead-Box Protein P3" OR foxp3 OR "immune homeostasis" OR immunsuppress*)*. For animal studies, the search string included synonyms for [1] physical exercise, [2] animal (i.e. rodent) population, and [3] T_{reg} characteristics: *(exercise OR fitness OR sport* OR training OR "wheel running" OR HIIT OR HIT OR cycling OR walking OR swimming OR running) AND (murine OR mouse OR mice OR rat OR rats OR rodent OR animal) AND ("regulatory T cells" OR Treg* OR "Forkhead-Box Protein P3" OR foxp3 OR CD25 OR "immune homeostasis" OR immunsuppress*)*.

For both search strategies, the search string was adjusted according to the formal requirements of each database and the scope of the search was restricted to peer-reviewed articles published in English. After deduplication and screening (title/abstract) by two reviewers (SP and MW), 33 human studies (34 animal studies) were independently assessed for full-text analysis. Through cross-referencing, ten additional human studies (one animal study) were included in the review process. Finally, 37 human studies (23 animal studies) were included in the systematic review (see figure 1 and figure 2).

Eligibility criteria

All types of acute or chronic exercise interventions, regardless of additional interventions (e.g. supplementation, medication), were considered eligible. Studies that did not report on group effects (animals) or group and/or time effects (humans) were excluded. For both the human and animal category, studies that examined the T_{reg} cell counts, proportion, and suppressive function in any tissue or body compartment were included. Further, studies that measured Foxp3 gene expres-

sion, which was linked to T_{reg} cells by the authors, were included as well. Studies with insufficient or unclear reporting on T_{reg} outcome measures were excluded. Human studies that used a randomized, non-randomized controlled, non-controlled, or cross-sectional design were eligible for inclusion. For animal studies, only randomized and non-randomized controlled trials were included. Case-studies, editorials, reviews, and conference abstracts were excluded.

Data extraction

The following data of included studies were extracted and summarized in table 2 and 3: first author and year of publication; sample size; detailed description of the intervention group, exercise protocol and control group; study design; time point of blood sampling (table 2 only); type of tissue used for analysis and time point of tissue sampling (table 3 only); characterization/phenotyping of T_{reg} population; T_{reg}-related outcome measures; T_{reg}-related results.

Quality assessment and Risk of Bias

Quality assessment of internal validity and risk of bias within included human studies were assessed with the Tool for the assEssment of Study qualiTy and reporting in EXercise (TESTEX) (127). The TESTEX scale contains 12 items and has been designed to assess the quality of reporting of exercise training studies. It covers eligibility criteria, random allocation, concealment of allocation, comparability of groups at baseline, blinding of assessors, reporting of study adherence/exercise attendance/adverse events, analysis by intention to treat, between-group statistical comparisons, point measures and variability data, activity monitoring in control group, constant relative exercise intensity, and reporting of exercise volume and energy expenditure. The TESTEX is not adequate for assessing the study quality of acute exercise interventions and there is no other study quality and reporting assessment tool for acute exercise studies. Therefore, acute studies were not rated.

Quality assessment of internal validity and risk of bias within included animal studies were assessed with the "Office of health assessment and translation risk of bias rating tool" – for Human and Animal Trials (OHAT) (157). The tool contains 11 Risk-of-bias questions that cover 6 different fields of biases (selection, confounding, performance, attrition/exclusion, detection, selective reporting). Eight of the eleven questions can be answered using one of four predefined answer choices that enable the researcher to categorize and quantify the outcome. The remaining three questions are closed-ended questions ("yes" or "no").

Both the quality and the risk of bias of each study were independently assessed by two reviewers (MW and NJ) with an intraclass inter-rater correlation coefficient of 94,56% (TESTEX) and 92,17% (OHAT), respectively. In case of disagreement between the two reviewers, a third reviewer (SP) was consulted.

Differences in T_{reg} phenotyping between studies represent an additional risk of bias. As it is recommended to stain at least for CD4, CD25 and Foxp3/CD127 to identify human and animal T_{regs} (112), considering that CD127 expression inversely correlates with FoxP3 expression (78), phenotyping of T_{regs} is categorized (see table 2 and 3) as sufficient (green color: at least CD4, CD25, Foxp3/CD127), insufficient (light red color: only CD4, CD25 or CD4, Foxp3) or highly insufficient (dark red color: Foxp3 gene expression in leukocytes or PBMCs).

Results

The search strategy for human studies led to 1610 results. Ten additional studies were identified via cross-referencing. After removing duplicates, the titles and abstracts of 1460 studies were screened for eligibility. The remaining 43 studies were assessed for a full-text screen. After applying the selection criteria, six studies were excluded (no/unclear reporting on Treg outcome measures, study protocol, review). Finally, 37 studies were included in the systematic review. The PRISMA flow diagram is provided in figure 1.

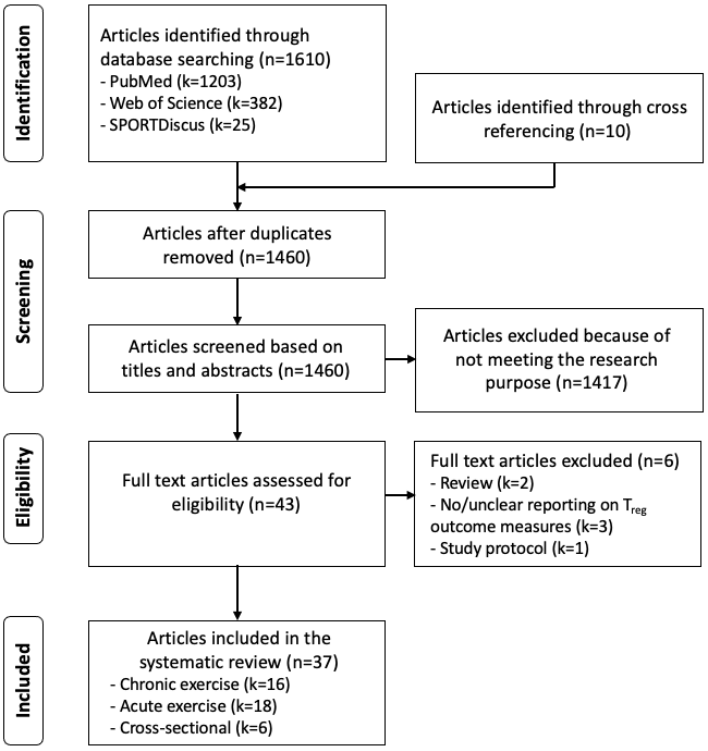

Figure 1: Literature search and results of human studies. Three studies (29, 48, 143) fit into the acute and chronic exercise category.

The search strategy for animal studies led to 1642 results. One additional study was identified via cross-referencing. After removing duplicates, the titles and abstracts of 1267 studies were screened for eligibility. The remaining 34 studies were assessed for a full-text screen. After applying the selection criteria, eleven studies were excluded (no exercise intervention, no/unclear reporting on T_{reg} outcome measures, T_{reg} knockout). Finally, 23 studies were included in the systematic review. The PRISMA flow diagram is provided in figure 2.

Characteristics of included studies

There is a tendency for an increased inclusion of T_{reg} outcome measures into human and animal studies in recent years. The overall sample size of included participants in the human chronic exercise studies is 619, with a mean intervention duration of 70.25 days (ranging from four days to six months). In the acute studies, a total of 398 participants performed either an "exercise-to-exhaustion" protocol [6], another intense [9] or a moderate training protocol [4]. The six cross-sectional

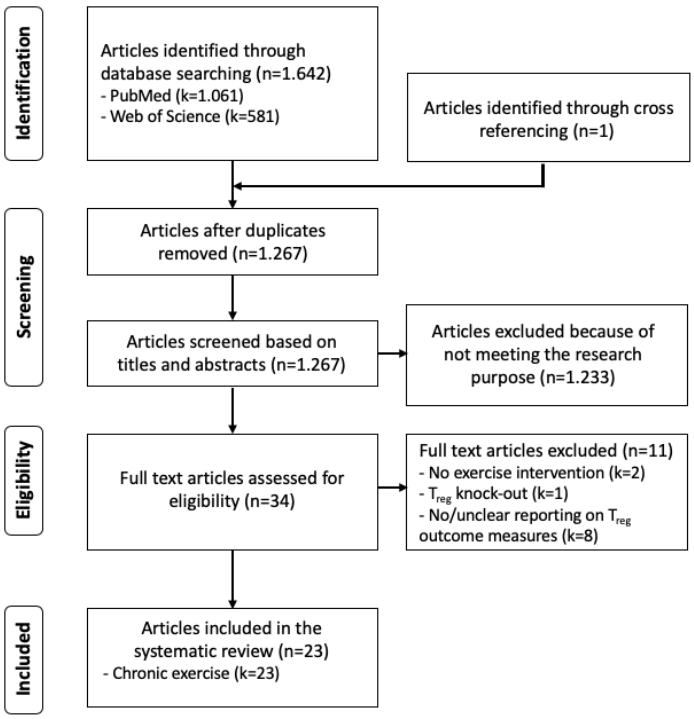

Figure 2: Literature search and results of animal studies.

studies comprise 745 predominantly healthy participants, although the participant characteristics strongly differ. There are only three studies that measured the single or combined effect of strength training on T_{regs}. Seventeen human studies collected data on cell counts, 31 studies on cell proportions, while only ten studies measured both markers. Three human studies measured cellular suppressive capacity either directly, in T_{regs} and peripheral blood mononuclear cells (PBMCs), or indirectly via Interleukin (IL)-10 production of whole blood. Another three studies solely identified Foxp3 gene expression in immune cells that the authors related to T_{reg} cells.

The overall sample size of included animals in the 23 animal studies (only chronic exercise interventions) is 713, with a mean intervention duration of 44.7 days (ranging from four days to twelve weeks). There is only one study that measured the single effect of strength training on T_{regs}. Concerning T_{reg} outcome measures, four animal studies collected data on cell counts, 18 on cell proportions, while two studies measured the suppressive capacity of tissue-specific T_{regs}. Two studies only identified Foxp3 gene expression in tissue homogenates that the authors related to T_{reg} cells.

Rating of bias

None of the included human studies fulfilled all the TESTEX quality criteria. No studies met item 6.2 (reporting of adverse events). Three items were fulfilled only by one study (intention-to-treat analysis (77), activity controlling in control group (77), exercise volume and energy expenditure (38)). On average, each study fulfilled 4,44 criteria representing 29,6% of all items. Figure S1 (supplement material) shows detailed information on each trial rating.

None of the included animal studies fulfilled all OHAT quality criteria. No study considered adequate concealment to study groups (increased selection bias) and outcome data completion without attrition or exclusion from analysis (increased attrition/exclusion bias). Regarding performance bias, only two studies adequately blinded research personnel to the study group (129, 133). One study did not fulfill exposure characterization (107), two studies did not fulfill outcome assessment (84, 107) (increased detection bias). All researchers adhered to the study protocol, but only 26,1% of all studies used appropriate statistical methods. Figure S2 (supplement material) shows detailed information on each trial rating.

Effects of exercise on T_{reg} cells in healthy people

Studies that examined the effect of acute and chronic exercise on T_{reg} measures in the healthy population focus on active/physically fit and inactive/physically unfit adults, active elderly adults (master athletes and octogenarians), and (elite) athletes. Of note, the majority (82%) of the acute studies included healthy people, whereas only 25% of the chronic studies tested healthy people. In the following sections, cross-sectional studies [5] are presented first, followed by acute [14] and chronic [4] studies.

Handzlik et al. (48) showed that a higher physical activity level correlated with an increased T_{reg} proportion and whole blood in vitro IL-10 production when comparing sedentary, recreationally active, sprint- and endurance-trained males. Although the activity level did not differ between the endurance- and sprint-trained groups, the IL-10 production was significantly higher in the endurance group. Dorneles et al. (29) recently showed that obese men with high cardiorespiratory fitness (CRF), characterized as peak oxygen uptake (VO_{2peak}), had higher proportions of $CD4^+CD25^{high}CD127^{low}$ T_{regs} and $CD4^+CD25^+CD39^+$ memory T_{regs} than lean men with low CRF. Within the lean and obese cohorts, the high CRF groups consistently showed higher T_{reg} proportions compared to the low CRF groups. In another cross-sectional study, Weinhold et al. (143) found an almost linear relationship between the relative VO_{2peak} level and the T_{reg} proportion in a heterogeneous cohort of 245 elite athletes. Moreover, both the T_{reg} proportion and suppressive function of T_{regs} from athletes were significantly higher compared to an age- and sex-matched healthy control group. Rehm et al. (110) showed that the T_{reg} proportion of marathon-trained runners near peak in training volume was decreased compared to demographically matched controls. This group difference disappeared when the body mass index was considered as a covariate. Another cross-sectional study of master cyclists (55-79 years) revealed no differences in the T_{reg} proportion compared to younger controls, while both groups showed lower Treg levels than the elderly age-matched control group (36).

Concerning the acute effects of exercise on T_{reg} measures, the aforementioned study of Handzlik et al. (48) further examined the influence of cycling for 60 minutes at 70% VO_{2max} on T_{reg} counts and the T_{reg} proportion within the endurance-trained subgroup. However, no alterations were observed. Three separate studies measured the effect of a single strenuous endurance exercise (marathon, half-ironman triathlon) on T_{reg} counts and proportions. Perry et al. (102) observed a significant decrease in both measures immediately and ten days after a half-ironman triathlon or a marathon race. A subsequent in vitro assay revealed an increased suppressive effect of the athletes´ post-exercise serum on T_{reg} proliferation compared to athletes´ pre-exercise serum. In response to a marathon race, similar changes in T_{reg} kinetics have been observed by Clifford et al. (21). However, the decrease of T_{reg} counts and propor-

tion one hour after the marathon raised above baseline levels after one day. The same post-exercise kinetic accounted for naïve CD45RA$^+$ T_{regs}, whereas the proportion of terminally differentiated HLA-DR$^+$ T_{regs} only increased one day after the marathon. Another study in marathoners revealed no changes in the T_{reg} proportion on day seven post-marathon compared to pre-race levels (111). In elite soccer players, treadmill running until exhaustion induced an increase in the T_{reg} proportion at 17 hours post-exercise, whereas no differences were observed immediately after exercise (66). In a parallel-group study with elite soccer players, the effect of two different exhaustive exercise protocols (YO-YO test vs. Beep test) on CD4$^+$ Foxp3$^+$ T_{regs} was examined (67). Following the YO-YO test, the T_{reg} proportion declined at 17 hours post-exercise, while there was a significant increase in T_{regs} immediately and 17 hours after the Beep test. Minuzzi et al. (88) tested the effect of cycling exercise until exhaustion on the proportions and cell counts of several CD4$^+$CD25^{++}CD127$^{-/low}$ T_{reg} populations in active master athletes (53.2 ± 9.08 years) and age-matched controls. An increase in T_{reg} counts ten minutes post-exercise and a concomitant return to baseline levels after one hour was observed in both groups. No changes in IL-10$^+$-, TGF-β$^+$-, Foxp3$^+$-expressing T_{regs}, naïve (CD45RA$^+$), memory (CD45RA$^-$), and terminally differentiated (KLRG1$^+$) T_{regs} were shown. Dorneles et al. (30) revealed an increase in the CD4$^+$CD25$^+$CD39$^+$ memory T_{reg} proportion in healthy men with low and high CRF immediately and one hour after a single high-intensity interval training (HIIT). Of note, T_{reg} proportions were significantly greater in high CRF compared to low CRF men at baseline. In a study on middle-aged women (64), no change in the T_{reg} proportion in response to cycling until exhaustion was shown. However, the participants´ CRF correlated positively with baseline T_{reg} levels. By using a randomized cross-over design of acute moderate resistance and endurance exercise, the group of Zimmer et al. (57, 121) showed no changes in CD4$^+$CD25$^+$CD127^{dim} T_{reg} counts and proportions in response to resistance exercise, but significant increases in T_{reg} counts immediately after the endurance exercise that was accompanied by a decrease to baseline levels one hour after exercise cessation. Another study conducted by Krüger et al. (69) compared the effect of acute moderate continuous exercise to HIIT in untrained healthy men by using a cross-over design as well. There was an increase in circulating T_{regs} only following HIIT, lasting for three hours, whereas a higher proportion of apoptotic Annexin V$^+$ T_{regs} was shown only in response to the moderate continuous exercise with a significant group difference three hours post exercise.

With respect to chronic effects of exercise on T_{reg} measures in healthy subjects, the aforementioned study of Weinhold et al. (143) conducted a one-week intense training intervention (German Olympic Hockey Team) and revealed a mean increase of 12,9% in the T_{reg} proportion. In two different studies, Yeh et al. (149, 152) focused on the effect of a long-term moderate exercise intervention on the T_{reg} compartment in middle-aged healthy adults. Both the twelve-week Tai chi chuan (149) and the gymnastic exercise intervention (152) led to an increase in T_{reg} counts. However, when compared to the passive control group, the results did not reach significance (152). Another study by Van Geest et al. (135) found that a strenuous endurance exercise (4-day walk, 30 km each day) in octogenarians (81.3 ± 1.9 years) increased naïve CD45RA$^+$ T_{reg} counts, whereas no change was observed in memory CD45RA$^-$ T_{regs}.

Effects of exercise on T_{reg} cells in pathological conditions

The included human studies comprise a wide spectrum of diseased population. Most of them are complemented by studies of animal disease models. In the following sections, studies on MS and cancer, as being the most investigated disease categories, are presented separately, followed by a section that comprises other disease categories. For each category, human studies are described first, followed by related animal studies.

In a cross-sectional study, Waschbisch et al. (141) found no difference in circulating CD4$^+$ CD25$^+$Foxp3$^+$ T_{regs} between physically active and inactive patients with relapsing-remitting MS. A subsequent incremental test to exhaustion revealed no association between VO_{2max} and the baseline T_{reg} proportion. The study of Deckx et al. (25) showed that twelve weeks of combined endurance and resistance exercise did not alter the T_{reg} proportion. In line with these results, a parallel-group study from Mähler et al. (80) observed no changes in the CD4$^+$CD25$^+$CD127$^-$ T_{reg} proportion in relapsing-remitting MS patients after 4 weeks of either normoxic or hypoxic endurance exercise. However, normoxic training led to a shift towards an increased proportion of CD39$^+$ T_{regs}. At the same time, a reduced CD31 expression, a marker for recent thymic emigrants, was observed. Hypoxic training led to the opposite effect with increased CD31 and reduced CD39 expression, respectively.

By using mice with induced experimental autoimmune encephalomyelitis (EAE) as the main MS model, Souza et al. (129) found a significant higher splenic CD4$^+$CD25$^+$ T_{reg} proportion at day 14 (strength and endurance training) and day 30 (strength training only) after EAE induction compared to EAE passive controls. Xie et al. (147) revealed that eight weeks of high-intensity swimming significantly increased CD4$^+$CD25$^+$Foxp3$^+$ T_{regs} in the central nervous system (CNS)- and lymph node compared to the moderate training and control groups. Results from Bernardes et al. (9) did not confirm these findings in EAE mice, as no group difference in T_{reg} counts or proportion in the spinal cord was observed after six weeks of swimming exercise.

In the context of cancer disease, Ligibel et al. (77) showed that, on average, four weeks (ranging from 10 to 70 days) of combined strength and aerobic exercise in invasive breast cancer patients did not change counts of tumor tissue-infiltrated Foxp3$^+$ leukocytes.

In two mammary tumor mouse models of 4T1 cell line injection, Hagar et al. (47) and Bianco et al. (10) performed analyses of T_{reg} measures in dissected tumor tissues. Hagar et al. used an immunohistochemical approach and showed significant reductions in CD4$^+$Foxp3$^+$ T_{regs} and an increase in the CD8/T_{reg} ratio compared to a passive control group. Bianco et al. identified no changes in mean fluorescent intensity of tumor-infiltrating CD4$^+$Foxp3$^+$ T_{regs} between tumor-trained and untrained mice. However, splenic CD4$^+$CD25$^+$ T_{regs} were decreased, while Foxp3$^+$ gene expression in splenocytes was increased in the exercise group. In a 7,12-dimethylbenzanthracene-induced mammary tumor model (1), an eight-week exercise intervention before tumor induction lowered the splenic T_{reg} proportion in tumor-trained mice compared to passive controls. When three weeks of moderate swimming exercise before and six weeks after liver tumor induction was applied,

reductions of the T_{reg} proportion in several tissues, i.e. tumor tissue, spleen, and blood, were observed. In a genetic model of intestinal tumorigenesis, McClellan et al. (84) revealed a decrease in Foxp3 gene expression in mucosal scrapings after a twelve-week running intervention.

In adolescent elite swimmers with and without allergic asthma, Wilson et al. (146) found that acute intense swimming exercise increased $CD4^+CD25^+$ and $CD4^+Foxp3^+$ T_{regs}, respectively, in both groups. However, no group difference for any cell population was reported. In response to 12 weeks of chronic moderate exercise, Liao et al. (76) revealed increases in the T_{reg} proportion in asthmatic children, but again, without a difference compared to passive controls. In three mouse models of allergic asthma, using an ovalbumin sensitization protocol beginning three weeks before exercise commencement, Fernandes et al. (40) found that five weeks of moderate exercise increased counts of $IL\text{-}10^+$ and LAP^+ (an important marker of TGF-β excretion in T_{regs}) T_{regs} in lung tissue of asthmatic but not healthy mice. Similar findings were shown for the T_{reg} proportion and suppressive capacity in lungs and lung-draining lymph nodes of asthmatic mice compared to passive controls after four weeks of exercise (79). Although no changes in the T_{reg} proportion was observed, a significant group difference was evident for T_{reg} suppressive function. Dugger et al. (37) adoptively transferred either wild type β2 adrenergic receptor (β2AR) T_{regs} or β2AR knockout T_{regs} into T_{reg}-depleted asthmatic mice. Results show that an adoptive transfer of wild-type $\beta2AR^+T_{regs}$ into mice prior to asthma induction increased cyclic AMP (cAMP) levels in splenic and lymph node T_{regs} in response to chronic exercise compared to mice with $\beta2AR^{-/-}$ T_{regs}. This increase in intracellular cAMP was associated with an enhanced cell-dependent suppressive function. The catecholamine-dependent increase in T_{reg} functionality was confirmed by elevations in the cAMP concentration due to β2AR agonist application.

Six months of intradialytic cycling did not change $CD4^+CD25^+CD127^{low/-}$ T_{reg} counts in haemodialysis patients (38). In the usual care control group, however, T_{regs} decreased below baseline levels which resulted in a significant group difference after the intervention period. In a mouse model of cisplatin-induced acute kidney injury, Miyagi et al. (117) found that five weeks of aerobic running exercise before disease induction did not affect $CD4^+Foxp3^+$ T_{reg} proportion in spleen and kidney lymph nodes, respectively, compared to passive controls. However, significantly lower levels in $CD4^+CD25^+$ (spleen and lymph nodes) were identified in response to exercise.

Yeh et al. showed that twelve weeks of Tai Chi Chuan increased both $CD4^+CD25^+$ T_{reg} counts and proportions in a non-controlled trial with diabetic adults (151). The same exercise protocol was used in a controlled trial which revealed an increased Foxp3 gene expression in leukocytes only in the diabetic cohort, however, without a group effect (150). Another study did not find any changes in the T_{reg} proportion in response to twelve weeks of moderate cycling (144). In a genetic mouse model of diabetes type II, six weeks of whole-body vibration exercise resulted in an increased proportion of circulating T_{regs} compared to passive controls (153).

Dorneles et al. (29) showed that one week of HIIT in obese men with low CRF increased both $CD4^+CD25^{high}CD127^{low}$ T_{reg} and $CD39^+$ memory T_{reg} proportions, correlating positively with exercise-induced increases in VO_{2peak}. However, in a study that tested eight weeks of running exercise on the T_{reg} proportion in diet-induced obese mice, no change was observed (74).

Barhoumi et al. (6) examined the effect of an eight-week swimming intervention on Foxp3 gene expression in renal cortex cells in a mouse model of hypertension and found that the exercise-induced increased expression was associated with lower erythropoietin-induced adverse vascular effects such as oxidative stress, inflammation, and immune activation. In a study using an atherosclerosis-prone mouse model, five weeks of running did not change the proportion of lymph node $CD4^+CD25^+Foxp3^+$ T_{regs} (56).

In a murine heart transplantation model (133), running exercise for one week after transplantation had the most beneficial effect on transplantation success compared to other exercise regimens and led to increases in the splenic T_{reg} proportion. When injecting whole splenocytes or $CD4^+$ splenocytes from this exercise group to heart-transplanted mice that did not exercise, significantly longer graft survival rates were observed compared to untreated mice. In line with these findings, Rael et al. (107) observed a beneficial effect of combined preceding (four weeks) and succeeding (one week) voluntary exercise on skin transplantation success. However, they did not find any changes in the $CD4^+Foxp3^+$ T_{reg} proportion in the skin allograft and even a decline in skin-draining lymph nodes.

Table 2: Human studies on the effect of exercise and physical activity on regulatory T cells. Phenotyping of T_{regs} is categorized as sufficient (green: at least CD4, CD25, CD127 or CD4, CD25, Foxp3), insufficient (light red: only CD4, CD25 or CD4, Foxp3) and highly insufficient (dark red: Foxp3 gene expression in leukocytes or PBMCs). Each section (chronic, acute, cross-sectional) is sorted by T_{reg} outcome measure (first proportion & counts, then proportion, then counts, then Foxp3 gene expression).

AUTHOR, YEAR	N	POPULATION, INTERVENTION GROUP	EXERCISE PROTOCOL	STUDY DESIGN, CG SPECIFICATION	TIMEPOINT OF SAMPLING	T_{REG} PHENO-TYPING	T_{REG} OUTCOME MEASURES	RESULTS
CHRONIC INTERVENTION STUDIES								
DUNGEY 2017 (38)	31 f+m	Haemodialysis patients; n=16	6 months of progressive intradialytic exercise on recumbent cycle at RPE 12–14 (offered thrice weekly)	CT / CG: same population; passive (usual care); n=15	Before and after intervention (at least 48h after last exercise)	$CD4^+ CD25^+ CD127^{low/-}$	Proportion (%$CD4^+$ cells), Counts (cells/µL)	↔ T_{reg} count post vs. baseline in exercise group; ↓ T_{reg} count post vs. baseline in control (p=.003); ↑ T_{reg} count in exercise vs. control group (p=.02); ↔ T_{reg} proportion in either group after intervention; No difference between groups
YEH 2007 (151)	32 f+m	Type 2 diabetic patients	12 weeks of Tai Chi Chuan exercise (classified as moderate exercise), 3 sessions/week à 60 min	NCT	Before and 3 days after intervention	$CD4^+ CD25^+$	Proportion (%$CD4^+$ cells), Counts (cells/mm^3)	↑ T_{reg} count after exercise (p=.029) ↑ T_{reg} proportion after exercise (p=.001)
DECKX 2016 (25)	45 f+m	MS patients (all types, EDSS ≤ 6); n=29	12 weeks of combined endurance/resistance exercise (5 sessions in 2 weeks, continuous progression of duration [1×6min/session to 3×10min/session] and intensity [1×10 rep. to 4×15 rep.])	RCT / CG: same population; passive; n=16	Before & within the week after intervention (overnight fast at 8 a.m.)	$CD4^+ CD25^{high} Foxp3^+$	Proportion (%$CD4^+$ cells)	↔ T_{reg} proportion in exercise and control group; No differences between groups
MÄHLER 2018 (80)	30 f+m	Relapsing-remitting MS patients (EDSS ≤ 4)	4 weeks of either normoxic (NO, n=16) or hypoxic (HP, corresponding to 2.500m altitude, n=14) treadmill exercise (3 sessions/week à 1h)	Pilot parallel-group RT	Before and after intervention (12h overnight fast)	$CD4^+ CD25^+ CD127^-$ (T_{regs}) + $Foxp3^+$, CD31, CD39, CD45RA on T_{regs}	Proportions of T_{regs} and all subpopulations (%T_{regs})	↔ T_{reg} proportion and any T_{reg} subpopulation in the HO group; ↑ $CD39^+ CD31^-$ T_{reg} proportion in the NO group; ↑ $CD39^+$ T_{reg} proportion in the NO group; ↓ $CD39^- CD31^+$ T_{reg} proportion in the NO group; ↑ $CD39^+$ $Foxp3^+ CD45RA^+$ T_{reg} proportion in the NO group; Significant group differences for $CD39^+ CD31^-$ T_{reg} / $CD39^+ Foxp3^+ CD45RA^+$ T_{reg} (higher in NO) and $CD39^- CD31^+$ T_{reg} (higher in HO) after exercise
DORNELES 2019 (29)	7 m	Low CRF obese men (VO_{2peak} of 34.54 ± 3.27 mL/kg/min)	1 week of HIIT-Training on treadmill (3 sessions à 10 bouts of 60 sec (85-90% HR_{max}), 75 sec recovery (50% HR_{max}) between bouts)	NCT	Immediately before, 24h after last session	$CD4^+ CD25^{high} CD127^{low}$ / $CD4^+ CD25^+ CD39^+$ (mT_{reg})	Proportion (%lymphocytes)	↑ T_{reg} proportion (p=.04) ↑ mT_{reg} proportion (p=.042)
WEINHOLD 2016 (143)	19 m	Olympic hockey team	Single 1-week intense training phase	NCT	Immediately before + after intervention (not specified)	$CD4^+ CD25^{high} CD127^{low}$	Proportion (%$CD4^+$ cells)	↑ T_{reg} proportion after intervention (p=.021)
DOS SANTOS 2015 (91)	60 f	Overweight (n=15) and eutrophic (n=15) pubescent children	18 weeks of circus activities in a circus school (2 sessions/ week à 60 min)	RCT / CG: same population; passive; n=2x15	No pre assessment; at least 48h after last exercise session	$CD4^+ CD25^+ Foxp3^+$	Proportion (% n.a.)	Lower T_{reg} proportion in overweight exercise vs. eutrophic exercise group (p<.05) and overweight control vs. eutrophic control group (p<.05); No difference between overweight exercise vs. control group
WENNING 2013 (144)	14 m	Type 2 diabetic patients	12 weeks of moderate aerobic cycling (2 sessions/week, progressively increasing from 15 to 45 min/session)	NCT	Before and after intervention (not specified)	$CD4^+ CD25^{++} CD127^{low}$	Proportion (%$CD4^+$ cells)	↔ T_{regs} proportion after intervention
LIAO 2019 (76)	40 f+m	Children (6-12y) with mild to severe persistent asthma; n=25	12 weeks of Tai Chi Chuan exercise (1 session/week à 60 min); specifically designed for asthmatic children	CT / CG: same population; passive; n=15	Before and after intervention (not specified)	$CD4^+ CD25^+ Foxp3^+$	Proportion (%$CD4^+$ cells)	↑ T_{reg} proportion in exercise group (p=.008); No differences between groups
WANG 2016 (140)	87 m	Heroin users; n=30	12 weeks of Baduanjin qigong training (5 sessions/week à 60min)	CT / CG: 2 groups; 1st group: same population, passive; n=30 2nd group: healthy men; n=27	Before and after intervention (not specified)	$CD4^+ CD25^+ Foxp3^+$	Proportion (%n.a.), Suppressive function of PBMCs (Thymidine H^3 in T_{Eff})	↓ T_{reg} proportion in heroin exercise group after vs. before intervention (p<.001); ↓ T_{reg} proportion in heroin exercise vs. heroin control group after intervention (p<.001); ↓ suppressive function in heroin exercise group (time and group effect, both p<.001))
YEH 2014 (152)	44 f	Inactive middle-aged women (Ø age: 47); n=22	12 weeks of regular music aerobic exercise (classified as moderate exercise), 3 sessions/ week à 60 min	CT / CG: same population; passive; n=22	Before and 30 min after last exercise session	$CD4^+ CD25^+$ / Foxp3 gene expression in leukocytes	Counts (n.a.)	↑ T_{regs} in exercise group (p<.01) ↑ Foxp3 gene expression in exercise group (p<.01); No differences between groups
YEH 2006 (149)	37 f+m	Middle-aged healthy adults (Ø age: 55)	12 weeks of Tai Chi Chuan exercise (classified as moderate exercise), 3 sessions/week à 60 min	NCT	Before and 3 days after intervention	$CD4^+ CD25^+$	Counts (cells/mm^3)	↑ T_{reg} count after intervention (p=.015)
VAN DER GEEST 2017 (135)	20 f+m	Octogenarian walkers (Ø age: 81)	Nijmegen Four Days Marches (30 km a day, for 4 consecutive days at self-selected pace, (4.0±0.7 km/h ≙ moderate intensity))	NCT	Before, after (within 10 min after exercise termination)	$CD4^+ CD25^+ Foxp3^{high} CD45RA^-$, $CD4^+ CD25^+ Foxp3^{low} CD45RA^+$	Counts (x10^9/L)	↔ $CD4^+ CD25^+ CD45RA^- Foxp3^{high}$ count after the march ↑ $CD4^+ CD25^+ Foxp3^{low} CD45RA^+$ count after the march (p<.001)
YEH 2009 (150)	60 f+m	Type 2 diabetic patients; n=30	12 weeks of Tai Chi Chuan exercise (classified as moderate exercise), 3 sessions/week à 60 min	CT / CG: age-matched healthy adults; active (same protocol); n=30	Before and 3 days after intervention period	Foxp3 gene expression (in leukocytes)	Fold change (%) in mRNA expression	↑ Foxp3 gene expression in diabetic patients after exercise (p=.026), % change not reported; No differences between groups
RAYGAN 2017 (108)	44 m	Asymptomatic ischemic heart	12 weeks of submaximal aerobic exercise (3 sessions/week à 40	RCT / CG: same population; active	Before (24h) and after (24h)	Foxp3 gene expression (in	Fold change (%) in mRNA	↑ Foxp3 gene expression after exercise intervention (p<.02)

		disease patients	min)	(routine regular physical activities); n=23	intervention period	PBMCs)	expression	↑ Foxp3 gene expression in exercise vs. control group (p=.012)
LIGIBEL 2019 (77)	49 f	Invasive breast cancer patients (no metastases); n=27	Multi-week (Ø 4 weeks, range 10-70 days) combined strength and moderate-intense aerobic exercise of 220 min/week	RCT / CG: same population; passive (mind-body exercise); n=22	Tumor tissue collection at baseline and surgical tumor excision	Foxp3 gene expression (in tumor sample infiltrated leukocytes)	Fold change (%) in mRNA expression	↓ $Foxp3^+$ gene expression in exercise group (x0.76) ↑ $Foxp3^+$ gene expression in control group (x2.86) No differences between groups (p=.08)
ACUTE INTERVENTION STUDIES								
MINUZZI 2017 (88)	29 f+m	Active master athletes (different disciplines, Ø age: 53); n=19	CPET to exhaustion on cycle ergometer (25 watts increase every 3 min)	CT / CG: healthy inactive body weight- and age-matched controls; active (same protocol); n=10	Before, 10 min post, 1h post	$CD4^+CD25^{++}$ $CD127^{-low}$ (T_{regs}) $CD45RA^{+/-}$ T_{regs}, $KLRG1^+$ T_{regs}, IL-10^+ T_{regs}, $TGF\beta^+$ T_{regs}, $Foxp3^+$ T_{regs}	Proportion (%lymphocytes, $CD4^+$, T_{regs}), Counts ($x10^3/\mu l$), Foxp3, TGF-β and IL-10 gene expression in T_{regs}	Time effects for both groups: ↑ T_{reg} count at 10min post exercise (p<.05) ↔ for T_{regs} proportion (%$CD4^+$ & lymphocytes) ↔ $CD45RA^+$- and $CD45RA^-$ T_{regs} ↔ KLRG1 expression on T_{regs} ↔ Foxp3, TGF-β and IL-10 gene expression in any group No differences between groups for any outcome measure
CLIFFORD 2017 (21)	17 f+m	Experienced runners	Marathon race	NCT	Before (in the week leading up to the marathon), 1h post, 1-day post	$CD4^+CD25^{++}$ $CD127^-$ $Foxp3^+$ (T_{regs}), $CD45RA^+$ T_{regs}, $HLA\text{-}DR^+$ T_{regs}	Proportion (%$CD4^+$ & %lymphocytes), Counts (cells/μL)	↓ T_{reg} proportion (% $CD4^+$) at 1h post vs. before; ↑ T_{reg} proportion (% lymphocytes) at 1-day post vs. before; ↓ T_{reg} count at 1h post and ↑ at 1-day post (both vs. before); ↓ $CD45RA^+$ T_{reg} proportion (both %$CD4^+$ cells and lymphocytes) and count 1h post vs. before; ↑ $CD45RA^+$ T_{reg} count and $HLA\text{-}DR^+$ T_{reg} proportion (both %$CD4^+$ cells & %lymphocytes) 1-day post vs. before; all outcomes p<.05
PERRY 2013 (102)	38 f+m	Trained triathletes/ marathoners	Marathon race (n=16) and half-ironman triathlon (n=22)	NCT	4 days before race, immediately after race, 10 days after race	$CD4^+CD25^+$ $Foxp3^+$	Proportion (%$CD4^+$), Counts ($x10^4$/ml)	↓ T_{reg} count immediately and 10 days after race vs. before (each p=.002) ↓ T_{reg} proportion immediately and 10 days after race vs. before (each p<.001)
HANDZLIK 2013 (48)	10 m	Recreationally active healthy university students	60min on cycle ergometer at 70% of VO_{2max}	NCT	Before, immediately post, 1h post	$CD4^+CD25^+$ $CD127^{low/-}$	Proportion (%lymphocytes, %$CD4^+$), Counts($x10^9$/ml)	↔ T_{reg} count and proportion at any timepoint
GUSTAFSON 2017 (45)	15 m	Healthy active (n=10) & in-active (n=5) men	45min on cycle ergometer at 60% of PPO	NCT	Before, 2-5min post	$CD4^+CD25^+$ $CD127^{low}$	Proportion (%$CD4^+$)	↓ T_{reg} proportion 2-5 min post exercise (p=.006) in whole population; no post-exercise subgroup difference reported
SCHLAGHECK 2020 (121)	24 m	Healthy, physically fit men	Acute bout of endurance (45 min at 60% of PPO) and resistance exercise (4 sets at 5 resistance machines; 8-10 reps at 70% of 1RM)	RCT (crossover design)	Immediately before, immediately post, 1h post	$CD4^+CD25^+$ $CD127^{dim}$	Proportion (%$CD3^+$), Counts (10^3/μL)	↔ T_{reg} count after resistance exercise at any timepoint, ↑ T_{reg} count immediately post endurance exercise vs. before (p<.001); ↓ T_{reg} count 1h post endurance exercise vs. immediately post (p<.009), ↔ T_{reg} proportion in any group at any timepoint
KRÜGER 2016 (69)	23 m	Untrained healthy males	Single HIIT (5 bouts, 3 min each at 90% of PPO; 3 min active break with no resistance) and continuous exercise session (70% of VO_{2max} for 30 min) on cycle ergometer	CT (crossover design)	Before, immediately post, 3h post, 24h post	$CD4^+CD25^+$ $CD127^-$ (T_{regs}), Annexin V^+ T_{regs}	Proportion of Annexin V^+ T_{regs}, Counts ($x10^9$/l) of T_{regs}	↑ T_{reg} count immediately and 3h after HIIT exercise (each p<.05), sig. group difference immediately post vs. continuous exercise, ↔ in continuous exercise group; ↑ proportion of Annexin V^+ T_{regs} at 3h after continuous exercise, sig. group difference at 3h post vs. HIIT, ↔ in HIIT group
HARBAUM 2016 (49)	26 f+m	Idiopathic Pulmonary Arterial Hypertension patients; n=16	CPET on cycle ergometer (10 watts every min until exhaustion)	CT / CG: healthy age- and BMI-matched controls; active (same protocol); n=10	Before, immediately post, 1h post	$CD4^+CD25^{high}$ $CD127^{low}$	Proportion (% n.a.), Counts ($x10^6$/l)	↔ T_{reg} proportion and count in either group after exercise; No difference between groups IPAH patients with a higher baseline T_{reg} proportion than control (p<.05)
PERRY 2012 (101)	8 f+m	Chronic lympho-cytic leukemia patients; n=4	CCL patients: 45-60min walking/running on treadmill; Athletes: 60-120min running; both with HF at 70% of HRR	CT; CG: healthy athletes; n=4	Before, immediately post, 1h post	$CD4^+CD25^{high}$ $Foxp3^+$	Proportion (% n.a.)	↔ T_{reg} proportion in either group immediately after exercise; ↓ T_{reg} proportion 1h post exercise in the CLL group (p=.017)
KOLIAMITRA 2019 (64)	19 f	Healthy, physically fit women, age > 50	CPET on cycle ergometer (25 watts increase every 2 min until exhaustion)	NCT	Immediately before, 1 min post	$CD4^+CD25^+$ $CD127^{dim}$	Proportion (%$CD4^+$)	↔ T_{regs} proportion after exercise
REHM 2013 (111)	19 f+m	Recreational marathoners	Marathon race	NCT	4 weeks before (baseline), 24-48h before (pre race), 1 week after (recovery)	$CD4^+CD25^{high}$ $Foxp3^+$	Proportion (%$CD4^+$)	↔ T_{reg} proportion at any timepoint
KOSTRZEWA-NOWAK 2018 (66)	14 m	Elite soccer players	CPET on treadmill until exhaustion (speed increase of 2 km/h every 3 min) each in autumn and spring under same conditions	NCT (longitu-dinal design)	Before, max. 5 min post, 17h post	$CD4^+$ $Foxp3^+$	Proportion (%$CD4^+$)	↑ T_{reg} proportion at 17h post vs. before and 5 min post exercise (each p<.001) in autumn ↑ T_{reg} proportion at 17h post vs. before and 5 min post exercise (each p=.001) in spring
KOSTRZEWA-NOWAK 2020 (67)	62 m	Elite soccer players	Progressive exercise until exhaustion (YO-YO test [YYRL1 protocol, n=31] vs. Beep test [max. multistage 20 m shuttle run], n=31)	Parallel-group NCT	Before, max. 5 min post, 17h post	$CD4^+$ $Foxp3^+$	Proportion (%lymphocytes)	↓ T_{reg} proportion at 17h post vs. before (p<.01) and 5 min post (p<.001) in the YO-YO group ↑ T_{reg} proportion at 5 min post and 17h post vs. before (both p<.001) in the Beep group
KOSTRZEWA-NOWAK 2019 (65)	3 m	Elite karate athletes	CPET on treadmill until exhaustion (speed increase of 2 km/h every 3 min)	NCT	Before, max. 5 min post, 17h post	$CD4^+$ $Foxp3^+$	Proportion (%$CD4^+$)	↑ T_{reg} proportion at 17h post vs. before exercise (p<.05)
DORNELES 2019 (30)	30 m	Healthy men with high (n=15) and low (n=15) physical fitness	Single HIIT session on treadmill (10 bouts of 60 sec (85-90% HR_{max}) with 75 sec of recovery (50% HR_{max}) between bouts)	NCT	Before, immediately post, 1h post	$CD4^+CD25^+$ $CD39^+$ (mT_{reg})	Proportion (%$CD4^+$)	↑ mT_{reg} proportion in both groups immedi-ately and 1h post vs. baseline; ↑ mT_{reg} proportion in high vs. low physical fitness group at all timepoints (including baseline)
JUSZKIEWICZ 2018 (59)	19 m	Professional rowers; n=10	Controlled 2000-m time trial on rowing ergometer (before and after a 6-week training camp with Spirulina extract or placebo supplementation)	RCT / CG: same population; active (same protocol); n=9	Immediately before, 1 min post, 24h post	$CD4^+CD25^+$ $CD127^-$	Counts (pg/ml)	↔ T_{regs} count in either group after 1st trial (before training camp) ↑ T_{reg} count at 1min vs. baseline and ↓ T_{reg} count at 24h vs. 1min after 2nd trial (after training camp) in placebo group

JUSZKIEWICZ 2019 (60)	20 m	Professional rowers; n=10	controlled 2000-m time trial on rowing ergometer before and after a 6-week training camp (with L-Theanine or placebo supplementation)	RCT / CG: same population; active (same protocol, placebo); n=10	Immediately before, 1 min post, 24h post	$CD4^+ CD25^+ CD127^-$	Counts (pg/ml)	↔ T_{reg} count at any timepoint in any group after 1st trial (before training camp); ↑ T_{reg} count at 24h post vs. baseline in both placebo and supplementation group after 2nd trial (after training camp)
WILSON 2009 (146)	22 f+m	Elite adolescent swimmers with allergic rhinitis or asthma	7 min "race-swimming" exercise	CT / CG: swimmers without allergic rhinitis or asthma; active (same protocol); n=10	Before and post-race swimming exercise (not specified)	$CD4^+ CD25^+$, $CD4^+ Foxp3^+$	Counts (cells/µl)	↑ $CD4^+ CD25^+$ count after exercise in both groups (p<.001); ↑ $CD4^+ Foxp3^+$ cell count after exercise in both groups (p<.001); No differences between groups for any outcome
CROSS-SECTIONAL STUDIES								
HANDZLIK 2013 (48)	40 m	Active and inactive healthy university students	n.a.	4 groups (each n=10): sprint, endurance, recreational, sedentary	Once in resting condition	$CD4^+ CD25^+ CD127^{low/-}$	Proportion (%lymphocytes, $CD4^+$),Counts ($x10^9$/ml), in vitro IL-10 production of whole blood culture	↓ T_{reg} proportion (%$CD4^+$) in sedentary compared with all other groups (p<.001); ↑ proportion (%lymphocytes) in endurance compared to sedentary (p<.05); No group differences in T_{reg} cell count; ↑ IL-10 production in endurance athletes compared to all other groups
WEINHOLD 2016 (143)	280 f+m	Elite athletes with differing $relVO_{2peak}$ values (low vs. intermediate [int.] vs. high); n=245	n.a.	Compared to age- and sex-matched healthy persons (n=35)	Once in resting condition	$CD4^+ CD25^{high} CD127^{low}$	Proportion (%$CD4^+$), Counts (cells/µl), Suppressive function of T_{regs} (proliferation of T_{Eff}(CFSE labeling))	↑ T_{reg} count in high VO_{2peak} athletes vs. int. (p=.04) and low (p<.0001), ↑ T_{reg} proportion in high VO_{2peak} athletes vs. int. (p<.0001) and low (p<.0001) athletes; ↑ T_{reg} proportion int. vs. low (p=.038), ↑T_{reg} proportion in athletes vs. control (p=.0001), ↑T_{reg} proportion in male athletes vs. female athletes (p=.002), ↑ suppressive function of athlete T_{regs} vs. control (p=.04)
DORNELES 2019 (29)	90 m	Healthy obese (n=45) and lean (n=45) men with VO_{2peak} between 25.30 and 58.8 mL/kg/min	n.a.	6 groups (each n=15): lean (low, moderate high CRF), obese (low, moderate high CRF)	Once in resting condition	$CD4^+ CD25^{high} CD127^{low}$ (T_{reg}), $CD4^+ CD25^+ CD39^+$ (mT_{reg})	Proportion (%lymphocytes)	↑ T_{reg} proportion in lean high CRF vs. lean low and lean mod. CRF; ↓ T_{reg} in obese low CRF vs. lean low CRF; ↑ T_{reg} in obese high CRF vs. obese and lean low CRF; ↑ mT_{reg} in lean high CRF vs. lean low and lean mod. CRF; ↑ mT_{reg} in lean mod. CRF vs. lean low CRF; ↑ mT_{reg} in obese high CRF vs. obese low CRF; ↑ mT_{reg} in obese high and obese mod. CRF vs. lean low CRF
DUGGAL 2018 (36)	255 f+m	Active master athletes (non-elite cyclists, age 55-79); n=125	n.a.	Compared to healthy inactive age-matched (n=75) & young (n=55) adults	Once in resting condition	$CD4^+ CD25^{high} Foxp3^+$	Proportion (%$CD4^+$)	↑ T_{reg} proportion in inactive old vs. master cyclists and inactive young (both p=.001), no difference between master cyclists vs. inactive young
REHM 2015 (110)	38 f+m	Healthy recreational marathoners; n=19	n.a.	Compared to demographically matched controls (n=19)	Once in resting condition	$CD4^+ CD25^{high} Foxp3^+$	Proportion (%PBMCs)	↓ T_{reg} proportion in marathoners vs. controls (p=.044)
WASCHBISCH 2012 (141)	42 f+m	Relapsing-remitting MS patients (EDSS ≤ 3.5)	n.a.	Physically active (n=21) vs. inactive (n=21) patients	Once in resting condition	$CD4^+ CD25^+ Foxp3^+$	Proportion (%$CD4^+$)	No difference between groups

Abbreviations: BMI: body mass index; CCL: Chronic lymphocytic leukemia; CFSE: Carboxyfluorescein succinimidyl ester; CG: control group; CPET: cardiopulmonary exercise testing; CRF: cardiorespiratory fitness; CT: controlled trial; EDSS: Expanded Disability Status Scale; f: female; HIIT: high intensity interval training; HR_{max}: maximal heart rate; HRR: heart rate reserve; IPAH: Idiopathic Pulmonary Arterial Hypertension; m: male; mRNA: messenger RNA; MS: multiple sclerosis; mT_{regs}: memory T_{regs}; n.a.: not available; NCT: non-controlled trial; nT_{regs}: naive T_{regs}; PBMCs: Peripheral blood mononuclear cells; PPO: peak power output; RCT: randomized controlled trial; RPE: Rate of Perceived Exertion; RT: randomized trial; T_{Eff}: $CD4^+$ $CD25^-$ effector T cells; TGF-β: Transforming growth factor β; T_{regs}: regulatory T cells; $VO_{2max/peak}$: maximal/peak oxygen consumption; ↑: significant increase/higher; ↓: significant decrease/lower; ↔: no significant change.

Table 3: Animal studies on the effect of exercise on regulatory T cells. Phenotyping of T_{regs} is categorized as sufficient (green: at least CD4, CD25, CD127 or CD4, CD25, Foxp3), insufficient (light red: only CD4, CD25 or CD4, Foxp3) and highly insufficient (dark red: Foxp3 gene expression in tissue homogenate). The table is sorted by T_{reg} outcome measure (first proportion & counts, then proportion, then counts, then Foxp3 gene expression).

AUTHOR YEAR	*N*	*ANIMAL, MODEL ORGANISM*	*EXERCISE PROTOCOL*	*STUDY DESIGN, CG SPECIFICATION*	*TISSUE SAMPLE*	*TIMEPOINT OF SAMPLING*	*T_{REG} PHENOTYPING, METHODS*	*T_{REG} OUTCOME MEASURES*	*RESULTS*
BER-NARDES 2016 (9)	47 f	C57BL/6 mice with induction of EAE (multiple sclerosis model); n=23	6 weeks of swimming exercise (30 min/day, 5 day/week with 7% of body weight); EAE induction at the beginning of the 5th week	RCT / CG: passive; n=24	Spinal cord	42 days after EAE induction (=32 days after intervention)	$CD4^+$ $CD25^+$ $Foxp3^+$ / FC	Proportion (% n.a.), Counts (n.a.)	No difference in T_{reg} count or proportion between exercise vs. control group
XIE 2019 (147)	72 f	C57BL/6 mice with induction of EAE (multiple sclerosis model); 2 groups, each n=24	8 weeks of moderate-intensity swimming (=MIT) (50 min/day, 5 day/week with 0% of body weight) or high-intensity swimming (=HIT) (50 min/day, 5 day/week with 4% of body weight); EAE induction after the completion of the 6th week	RCT / CG: passive; n=24	CNS (brain, spinal cord), Lymph nodes	14 days after EAE induction	$CD4^+$ $CD25^+$ $Foxp3^+$ / FC	Proportion (%$CD4^+$)	↑ CNS T_{reg} proportion in HIT vs. MIT ($p<.05$) and control group ($p<.05$); ↑ Lymph node T_{reg} proportion in HIT vs. MIT ($p<.05$) and control group ($p<.01$); No difference of CNS or lymph node T_{reg} proportion between MT and control group
SOUZA 2017 (129)	12 f	C57BL/6 mice with induction of EAE (multiple sclerosis model); 2 groups, each n=6	4 weeks of strength training (=ST) (5 sessions/week à 30 min; climbing a ladder, carrying load that increases from 25-75% of body weight) or endurance training on a treadmill (=ET) (5 sessions/week à 30min; intensity of 13–17 m/min); EAE induction after completion of the 2nd week	RCT / CG: 2 groups; passive; 1st group: naive 2nd group: EAE each n=6	Spleen	7, 14 and 30 days after EAE induction	$CD4^+CD25^+$ / FC	Proportion (%spleno-cytes)	↑ T_{reg} proportion in ST vs. EAE control group at day 14 ($p<.001$) and 30 ($p<.05$); ↑ T_{reg} proportion in ET vs. naive control group at day 14 ($p<.001$) No difference of T_{reg} proportion between ST and ET at any timepoint
BIANCO 2017 (10)	40 f	BALB/c mice with/without mammary tumor induction (injection of 4T1 cell line); 2 groups, each n=10	4 weeks of swimming exercise after tumor induction (5 sessions/week; progression from 15 min in 1st week to 30 min in 2nd week to 45 min in weeks 3+4)	CT / CG: 2 groups; passive; each n=10	Spleen, Tumor tissue	On last exercise day (not specified)	$CD4^+$ $CD25^+$ / FC $Foxp3^+$ gene expression / PCR	Proportion (%$CD4^+$), Fold change of $Foxp3^+$ splenocytes, MFI of tumor infiltrating lymphocytes	↓ splenic T_{reg} ($p<.005$) proportion in tumor trained vs. control group; no differences between healthy groups; ↑ splenic $Foxp3^+$ gene expression in tumor trained vs. control group ($p<.03$); no differences in MFI of tumor infiltrating $CD4^+$ $Foxp3^+$ cells
ZHANG 2016 (154)	12 m	C57BL/6 mice with liver tumor induction (injection of Hepa 1-6 cell line); n=6	9 weeks of moderate swimming exercise (5 sessions/week à 5 min in 1st week, 6 min in 2nd week and 8 min in weeks 3-9); liver tumor induction at the end of week 3	CT / CG: passive; n=6	Whole blood, Spleen, Tumor tissue	Day 42 after tumor cell inoculation	$CD4^+$ $CD25^+$ $Foxp3^+$ / FC	Proportion (%$CD4^+$)	↓ T_{reg} proportion in blood, spleen and tumor tissue in exercise vs. control group (all $p<.05$)
ABDALLA 2013 (1)	56 f	BALB/c mice with/ without mammary tumor induction (via oral 7,12-DMBA); each n=14	8 weeks of swimming exercise after tumor induction (5 sessions/week à 45 min)	CT / CG: 2 groups; passive; each n=14	Spleen	After the intervention period; no specification	$CD4^+$ $CD25^+$ / FC	Proportion (%$CD4^+$)	↓ $CD4^+$ $CD25^+$ proportion in tumor trained vs. control group ($p<.05$); no differences between healthy groups
DOS SANTOS 2019 (32)	66 f	Normolipidemic/diet-induced hyper-lipidemic C57BL/6 mice with/without tumor induction (injection of B16F10 melanoma cells); 4 groups, each n=8	10 weeks of moderate training on treadmill (5 sessions/week à 1h at 45-55% of maximal speed, 5° incline); melanoma induction in 8th week	RCT / CG: 4 groups; passive; n=8/9/8/9	Mesenteric lymph nodes	21 d after melanoma cell or PBS injection (=shortly after intervention)	$CD4^+$ $CD25^+$ $Foxp3^+$ / FC	Proportion (%$CD4^+$)	No difference of T_{reg} proportion between any group ↓ T_{reg} proportion in hyperlipidemic control vs. normolipidemic control mice
MIYAGI 2018 (117)	38 m	C57BL/6 mice with (n=11)/without (n=10) cisplatin-induced nephrotoxicity	5 weeks of aerobic treadmill exercise before AKI induction (5 sessions/ week at 10/15/20 m/min; progression from 30 to 60 min in 1st week; 60minin weeks 2-5)	CT / CG: 2 groups; passive; n=8/9	Spleen, Kidney lymph node	5 days after AKI induction (=3 days after intervention period)	$CD4^+Foxp3^+$, $CD4^+$ $CD25^+$ / FC	Proportion (%$CD4^+$)	↓ splenic ($p<.01$) and lymph node ($p<.05$) $CD4^+$ $CD25^+$ cell proportion in AKI exercise group vs. control group; no difference in $CD4^+$ $Foxp3^+$ proportion in exercise AKI vs. control group
CHEN 2018 (18)	60 m	Sprague Dawley rats with induced ischemic cardiomyopathy; n=20	12 weeks of treadmill exercise after induction of ischemic cardiomyopathy (5 sessions/week à 30 min at 12m /min, 0° incline)	RCT / CG: 2 groups; 1st group: passive; 2nd group: sham passive;each n=20	PBMCs	post intervention (after overnight fast)	$CD4^+$ $CD25^+$ $Foxp3^+$ / FC	Proportion (%$CD4^+$)	↑ T_{reg} proportion in exercise vs. passive control group ($p<.05$) ↓ T_{reg} proportion in exercise and passive control group vs. sham passive control group ($p<.01$)
JAKIC 2019 (56)	48 f	Young/old C57BL/6 mice with/without high cholesterol diet; 4 groups, each n=6	5 weeks of treadmill exercise (5 sessions/week à 1h at 16 m/min, 5° incline)	CT / CG: 4 groups; passive; each n=6	Lymph nodes - inguinal - brachial - axillary	2 d after last exercise session	$CD4^+$ $CD25^+$ $Foxp3^+$ / FC	Proportion (%lympho-cytes)	No effect of exercise on lymph node T_{reg} proportion in any group; No difference in T_{reg} proportion between the groups
JAKIC 2019 (56)	48 f	Young/old C57BL/6 $ApoE^{-/-}$ mice (prone to atherosclerosis) with/without high cholesterol diet; 4 groups, each n=6	5 weeks of treadmill exercise (5 sessions/week à 1h at 16 m/min, 5° incline)	CT / CG: 4 groups; passive; each n=6	Lymph nodes - inguinal - brachial - axillary	2 d after last exercise session	$CD4^+$ $CD25^+$ $Foxp3^+$ / FC	Proportion (%lympho-cytes)	No effect of exercise on lymph node T_{reg} proportion in any group; No difference in T_{reg} proportion between the groups
LE GARF 2019 (74)	24 f	Diet-induced obese C57Bl/6J mice with/ without PPAR-β/δ agonist; each n=6	8 weeks of treadmill exercise (3 sessions/week; progression from 20 min (20 cm/s) in 1st week to 45min (30 cm/s) in week 8); begin of intervention: change to normal diet	CT / CG: passive; each n=6	Lymph nodes - inguinal - brachial - cervical	After intervention period (not specified)	$CD4^+$ $Foxp3^+$ / FC	Proportion (% n.a.)	↑ T_{reg} proportion in exercise + PPAR-β/δ agonist vs. control group; No difference between only exercise vs. control group
YIN 2015 (153)	12 m	Diabetic db/db and Non-diabetic db/m mice; 2 groups, each n=6	6 weeks of whole-body vibration exercise (5 sessions/week à 20 min at 30Hz (intensity not specified))	CT / CG: 2 groups; passive; each n=6	Whole blood	After intervention (not specified)	$CD4^+$ $Foxp3^+$ / FC	Proportion (%$CD4^+$)	↑ $Foxp3^+$ cell proportion in diabetic exercise vs. control group ($p<.05$); No group difference of $Foxp3^+$ cell proportions in non-diabetic mice
SONG 2019 (128)	n.a. n.a.	Healthy C57BL/6 mice; n=n.a.	30 consecutive days of whole-body vibration exercise (30 min/day: 10 min at 13Hz (moderate), 20 min at 17Hz (high intensity))	CT / CG: passive; n=n.a.	Spleen	After intervention period (not specified)	$CD4^+$ $CD25^+$ $Foxp3^+$ / FC	Proportion (%spleno-cytes)	↑ T_{reg} proportion in exercise vs. control group ($p<.01$)
RAEL 2019 (107)	20 f+m	C57BL/6 B6 mice (tail skin transplant, male B6 donors & female B6 recipients); n=10	4 weeks of voluntary wheel-running prior to skin transplantation with continuation of exercising thereafter for 10 days	CT / CG: same population; passive; n=10	Skin-draining lymph nodes, Skin allograft	10 days after transplan-tation	$CD4^+$ $Foxp3^+$ / FC	Proportion (%$CD4^+$)	↓ T_{reg} proportion in lymph nodes in exercise vs. control group ($p<.01$) No group difference of T_{reg} proportion in skin allograft

UCHIYAMA 2015 (133)	15 m	Heart transplanted CBA mice; n=5	7 days of endurance training on a treadmill after transplantation (1h/day at 9.6 m/min on 1st day and 12.8 m/min on days 2-7, 5° incline).	RCT / CG: 2 groups; 1st group passive; 2nd group: no operation (nOP); each n=5	Spleen, cardiac allograft tissue	1, 2 and 4 weeks after transplantation (spleen), 4 weeks after transplantation (cardiac allograft)	$CD4^+$ $CD25^+$ $Foxp3^+$ (T_{regs}) / FC $CD4^+$ $Foxp3^+$ / IHC	Proportion (%$CD4^+$)	↑ splenic $CD4^+$ $CD25^+$ $Foxp3^+$ proportion after week 1, 2 and 4 in exercise vs. passive and nOP group (all p<.001) except for proportion in exercise vs. passive group after 1 week; ↑ allograft infiltration of $CD4^+$ $Foxp3^+$ cells after 4 weeks in exercise vs. passive control group
WALKER 2018 (137)	12 n.a.	C57BL/6 Foxp3 reporter mice; n=6	4 days of voluntary wheel running	CT / CG: same population; passive; n=6	Spleen, Bone marrow, lymph nodes	n.a.; presumably 1 day after intervention period	$CD4^+$ $Foxp3^+$ (T_{reg}), $CD44^{+/-}$ $CD62L^{+/-}$ T_{reg}, $CXCR4^{+/-}$ T_{reg}, $CCR9^{+/-}$ T_{reg} FC	Proportion (%$CD4^+$, $Foxp3^+$ T_{regs})	↑ in splenic $CD4^+$ $Foxp3^+$ $CD44^+$ $CD62L^-$ proportion in exercise vs. control group (p<.05); No group differences of any cell proportion in lymph nodes or bone marrow; No group differences in T_{reg} chemokine receptor expression
WANG 2012 (139)	40 f	Healthy C57BL/6 mice; 2 groups, each n=8	6 weeks of moderate training (VO_{2max}~70%) or HIIT (VO_{2max}~91%) on a treadmill (5 sessions/week; moderate: week 1: 30 min at 8–18 m/min, week 2-6: 60 min at 18 m/min; HIIT: week 1: 30 min at 5m/min, week 2: 60 min at 15-26.8 m/min, week 3-6: 60 min at 26.8 m/min, 5-10° incline)	RCT / CG: 2 groups; 1st group: moderately active (5 m/min, 5 min/ day, 5 days/ week); n=8 2nd group group: "naïve": free exercise; n=8	Spleen	16-20h after intervention period	$CD4^+$ $CD25^+$, $CD4^+$ $Foxp3^+$ / FC	Proportion (%$CD4^+$)	↑ proportions of both cell types ($CD4^+$ $CD25^+$, $CD4^+$ $Foxp3^+$) in HIIT vs. moderate exercise, control and naïve group (all p<.05), no difference between the other groups; no difference between the other groups
LOWDER 2010 (79)	n.a. f	OVA-sensitized BALB/cj (asthma model) and healthy Foxp3 reporter mice; 2 groups, n=n.a.	4 weeks of moderate training on treadmill (3 sessions/week; 10.0 m/min for 30min to 13.5 m/min for 45min in 1st week, 13.5 m/min for 45min in weeks 2-4, 0° incline); begin of OVA-sensitization 3 weeks before intervention period	RCT / CG: 2 groups; passive; n=n.a.	Lung, Mediastinal lymph nodes, Spleen	24h after last exercise bout	$CD4^+$ $CD25^+$ $Foxp3^+$ / suppression assay $CD4^+$ $Foxp3^+$ / FC	Proportion (% n.a.), Suppressive function of $CD4^+$ $CD25^+$ $Foxp3^+$ (Thymidine H^3 in T_{Eff})	↑ $CD4^+$ $Foxp3^+$ proportion in lung & lymph node in OVA-exercise vs. control group (p=.01); no differences between healthy exercise vs. control group; ↑ suppression capacity of splenic, lung and lymph node $CD4^+$ $CD25^+$ $Foxp3^+$ in OVA-exercise vs. control group (p<.01); same results for healthy exercise vs. control group
FERNANDES 2019 (40)	40 m	OVA-sensitized (asthma model) and healthy BALB/c mice; 2 groups, each n=10	5 weeks of moderate training on treadmill (5 sessions/week à 1h at 50% of maximal speed); begin of OVA-sensitization 3 weeks before intervention period	CT / CG: 2 groups; passive; each n=10	Lung	1 d after intervention	$CD4^+$ $CD25^+$ $Foxp3^+$ LAP^+, $CD4^+$ $CD25^+$ $Foxp3^+$ $IL\text{-}10^+$ / FC	Counts (x10^6/n.a.)	↑ of both LAP^+ and $IL\text{-}10^+$ T_{reg} count in OVA-exercise vs. control group (p<.001); No group effect in either LAP^+ or $IL\text{-}10^+$ T_{reg} count in healthy mice; No difference in T_{reg} count between OVA and healthy mice
HAGAR 2019 (47)	16 f	BALB/c mice with mammary tumor induction (injection of 4T1 cell line); n=7	8 weeks of wheel-running before tumor induction (5 sessions/week; acclimatization in weeks 1+2 with zero to low intensity; 8 min/session in 2nd week to 26 min in week 8)	Pilot RCT / CG: passive; n=9	Tumor tissue	Euthanasia after tumor volume exceeded 1000mm^3	$CD4^+$ $Foxp3^+$ / IHC	Counts (per mm^2)	↓ T_{reg} count in exercise vs. control group ↓ T_{reg}/$CD8^+$ ratio in exercise vs. control group (p=.024)
BARHOUMI 2014 (6)	16 m	Wild-type and transgenic eET-1 overexpressing mice (hypertension model) with/ without EPO; n=7	8 weeks of swimming (6 sessions/ week à 60min with continuous progression in 1st week from 10 to 60 min [+10 min each day])	CT / CG: passive; n=9	Renal cortex	Immediately after the last exercise session	$Foxp3^+$ renal cortex cells/ Immunofluorescence microscopy	Counts (cells/mm^2)	↑ $Foxp3^+$ cell count in $eET\text{-}1^+$ exercise vs. control group (p<.05); ↑ $Foxp3^+$ cell count in $eET\text{-}1^+$ EPO exercise vs. control group (p<.05)
MCCLELLAN 2014 (84)	15 m	$Apc^{Min/+}$ mice (model of intestinal tumorigenesis); n=6	12 weeks of treadmill exercise (6 sessions/week à 1h at 15 m/min)	RCT / CG: passive; n=9	Mucosal tissue of small/large intestine	After intervention period (not specified)	$Foxp3$ gene expression in mucosal scrapings/ PCR	Fold change $Foxp3$ expression (in %)	↓ $Foxp3$ expression in exercise vs. control group (p<.05)

Abbreviations: AKI: acute kidney injury; CG: control group; cpm: counts per minute; CNS: Central nervous system; CT: controlled trial; DMBA: dimethylbenzanthracene; EAE: experimental autoimmune encephalomyelitis; eET-1: endothelial-specific endothelin-1; EPO: Erythropoietin; f: female; FC: Flow cytometry; HIIT: high intensity interval training; IHC: Immunohistochemistry; m: male; MFI: mean fluorescence intensity; n.a.: not available; OVA: Ovalbumin; PBMCs: Peripheral blood mononuclear cells; PBS: Phosphate-buffered saline; PCR: Polymerase chain reaction; RCT: Randomized controlled trial; T_{Eff}: $CD4^+$ $CD25^-$ effector T cells; T_{reg}: regulatory T cell; VO_{2max}: maximal oxygen consumption; ↑: higher values; ↓: lower values.

Discussion of results and future perspectives

This is the first systematic review that examined the effect of exercise-induced changes in T_{reg} outcome measures in human and animal studies, respectively. Taken together, higher CRF levels and a non-obese state are associated with an increased peripheral T_{reg} proportion in both species (29, 32, 64, 74, 91, 143). As T_{regs} are implicated in tumor progression, it needs to be elucidated whether these observations are also true for cancer patients. Acute bouts of exercise tend to either increase or to not change circulating T_{regs} post-exercise in humans (30, 48, 49, 59, 60, 64, 67, 69, 88, 121, 146), while strenuous endurance exercise such as a marathon race reduces T_{reg} levels (21, 102). With respect to T_{reg} kinetics during the recovery phase, levels tend to increase or remain constant rather than being decreased (30, 48, 49, 60, 65–67, 69, 88). However, this might not be the case in patients that suffer from cancer where a decline in T_{reg} levels has been observed (101). It is important to note that there are no acute exercise studies in animals. Only three long- (twelve weeks) (149, 151, 152) and two short-term (one week) (30, 143) chronic exercise interventions in humans showed increases of T_{reg} counts or proportion, whereas the remaining eight studies did not observe any changes in the peripheral T_{reg} compartment.

It is important to note that these three long-term studies (149, 151, 152) were conducted by the same research group that used the same moderate training protocol. With respect to animal studies, only three studies did not observe any difference in T_{reg}-related outcome measures (9, 32, 56), while seven studies reported reduced T_{reg} levels (1, 10, 47, 84, 107, 117, 154). Five out of the seven studies were mouse tumor models, thus a decline in T_{reg} counts or proportion is associated with a better clinical outcome. The remaining twelve studies revealed an increase in tissue-specific or circulating T_{regs}.

In the following sections, Treg assessment and study heterogeneity is discussed first, followed by discussions of study results in the context of current concepts in exercise immunology, i.e. T_{reg} differentiation, redistribution/apoptosis, and immunosenescence.

T_{reg} assessment

T_{reg} outcome measures often vary in the way how counts (e.g. 10^9/L, 10^4/ml, 10^3µl, cells/mm^3) and proportions (% of $CD4^+$/ $CD3^+$/ lymphocytes/ leukocytes) are presented. Moreover, T_{reg} phenotyping ($CD4^+CD25^+$, $CD4^+Foxp3^+$, $CD4^+CD25^+Foxp3^+$, $CD4^+CD25^+CD127^-$, Foxp3 gene expression) often differs between studies. Recently, a consortium of leading experts discussed existing human T_{reg} markers and, after validating marker combinations, agreed on a rationally composed ranking list of markers (119). Therefore, it is highly recommended to adhere to the following panel for proper (basal) T_{reg} characterization: CD4+, CD25+, and Foxp3/$CD127^{low}$. When further phenotyping of T_{reg} subsets was done in the included studies, $CD39^+$, $CD45RA^-$, $CD45RO^+$, $CD28^+CD57^-$, or $CD62L^-CD44^+$ T_{regs} were categorized as memory T_{regs}, while $CD45RA^+$, $CD45RO^-$, or $CD62L^+CD44^-$ T_{regs} were used to specify naïve/low differentiated T_{regs}. Recent thymic emigrants (RTEs) were identified by being either $PTK7^+$ or $CD31^+$, while $CD28^-CD57^+$ or $KLRG1^+$ T_{regs} were characterized as senescent or highly/terminally differentiated T_{regs}. The high variation in the used subset markers makes it difficult to adequately compare study results.

Some studies only measured T_{reg} counts without assessing the T_{reg} $CD4^+$ proportion which may be of limited significance when similar changes occurred in the overall $CD4^+$ T cell population after acute or chronic exercise. For studies that only measured the T_{reg} proportion relative to lymphocyte or leukocyte counts, interpretation of changes in the T_{reg} proportion can be misleading if there are concomitant exercise-induced increases or decreases in the overall lymphocyte or leukocyte compartment. Exercise-induced mobilization differs within the major leukocyte subtypes and is characterized by a more profound mobilization of $CD8^+$ T-cells, Natural Killer (NK) cells, γδ T-cells, and neutrophils (126). In contrast, the $CD4^+$ compartment shows a less intense response. Therefore, it should be of interest to consider both measures, T_{reg} counts and the T_{reg} proportion relative to $CD4^+$ cells to guarantee an appropriate interpretation of results. A further assessment of T_{reg} functionality, either by staining for functionality-associated cytokines (e.g. IL-10, TGF-β), suppressive markers (CTLA-4), or by conducting functional assays to determine the contact-dependent/independent suppressive capacity (54), gives a more reliable indication of clinically important improvements. It should be taken into account that an increase in cell counts does not necessarily reflect an increase in the overall functional capacity of the cell population. For example, a current meta-analysis from our research group on the effect of acute exercise on the NK cell cytotoxic activity in healthy adults revealed a significant and intensity-dependent increase, but the improved function was not associated with an increased number of circulating NK cells (116).

Heterogeneity

There is a large heterogeneity regarding population, exercise protocols, and either blood sampling time points in human studies or time point of disease induction in animal models (see table 2 and 3). Therefore, our initial aim to conduct a meta-analysis was discarded. For acute studies, blood was not taken immediately, namely within the first minute after exercise cessation, in seven out of the 18 studies (21, 45, 65–67, 88, 111), but at five, ten, or 60 minutes after exercise. There is even more variability within recovery measurement time points, as blood samples were taken one hour (21, 30, 48, 49, 88, 101, 121), three hours (69), 17 hours (65–67), 24 hours (21, 69), seven days (111), and ten days (102) post-exercise. Although three studies tested a relatively homogeneous population with a similar exercise protocol (21, 102, 111), measurement time points at baseline, post and recovery were highly heterogeneous which impedes comparison of results.

Similar heterogeneity was found for chronic human studies. The post-intervention time point of blood sampling differed between 10 minutes (135), 30 minutes (152), 24 hours (29, 108), at least two days (38, 91), and three days (149–151). It is not recommended to take blood samples shortly after the final training session since cytokines and cellular immune components need approximately 24 hours to return to baseline levels. Of note, the remaining seven chronic studies did not report on the time point of sampling. This inconsistency further impedes a comparison between study results and hampers research reproducibility. Thus, more research with varying exercise modalities and intensities in different populations is needed to gain knowledge on modality- and intensity-depen-

dent chronic adaptions in the T_{reg} compartment.

With respect to animal studies, relevant differences between study designs need to be taken into account when interpreting results. In the studies of EAE mouse models (9, 129, 147), disease induction was consistently done two weeks before exercise cessation. However, Xie et al. (147) applied six weeks of exercise before EAE induction, while Bernardes et al. (9) applied four weeks and Souza et al. (129) two weeks of exercise. Concerning cancer studies, Hagar et al. (47) trained mice eight weeks before breast tumor induction, whereas Bianco et al. (10) applied four weeks of exercise training before induction in the same mouse model. Zhang et al. (154) tested the effect of three weeks of moderate swimming exercise before liver tumor induction, while McClellan et al. (84) used a genetic model of intestinal tumorigenesis so that no exercise was possible before disease onset. Of note, studies that investigated the effects of exercise in asthmatic mouse models (37, 40, 79), only small variations were evident regarding exercise protocol and disease induction. This homogeneity was also seen in the results, as all studies showed improved function and increased T_{reg} counts or proportion in asthmatic mice.

Exercise-induced T_{reg} differentiation

Currently there are two different concepts for exercise-induced T_{reg} differentiation. First, we highlight the kynurenine (KYN)-/kynurenic acid (KYNA)-dependent T_{reg} differentiation hypothesis that is supplemented by an intertwined TGF-β signalling mechanism. Then we discuss another, mainly cell metabolism-focused T_{reg} differentiation hypothesis.

The involvement of KYN- and KYNA dependent pathways in T_{reg} differentiation was delineated in the last issue of this journal (57). The interconnection between the tryptophan catabolism and T_{reg} generation via the rate-limiting enzyme, indolamin-2,3-dioxygenase (IDO) 1, has been confirmed. There is now strong evidence that KYN and KYNA or other aryl hydrocarbon receptor (AhR) agonists can induce T_{reg} differentiation (87, 148). Schlagheck et al. found an increase in circulating T_{regs} immediately after moderate endurance exercise that was followed by a decline to baseline levels after one hour (121). This was in line with the KYNA kinetics after endurance exercise. However, neither KYN levels nor the KYN-to-tryptophan ratio increased in response to the endurance exercise. Interestingly, the T_{reg} proportion significantly correlated with IDO1 implying an enhanced conversion from tryptophan to KYNA via KYN (57). In contrast, exhaustive exercise provoked the opposite response by increasing the KYN levels and KYN-to-tryptophan ratio, whereas no changes in T_{reg} levels immediately after exercise were observed (64). According to these results, it was suggested that repeating acute exercise-induced increases in KYN or KYNA might lead to a long-term increase in T_{regs}. However, no study so far examined the influence of exercise on cytoplasmatic or nucleic acid level (trans)differentiation pathways, e.g. KYN-AhR binding and translocation to the nucleus, within the $CD4^+$ T cell population. Since plasmacytoid dendritic cells play an important role in the KYN/KYNA-to-T_{reg} axis (17, 104), it may be interesting to include analyses of these cells into future studies. Deckx et al. (25) measured both T_{regs} and dendritic cells after twelve weeks of combined exercise in MS patients and found no changes in T_{regs} while circulating activated $CD80^+CD62L^+$ plasmacytoid dendritic cells increased. Similar results were found in the lymph nodes of asthmatic mice in response to chronic exercise (40). Future research should focus on cell culture approaches of combined cell lines in regards to tryptophan degradation, KYN/KYNA production and transport kinetics, SLC7A5 expression (KYN transporter) on naïve $CD4^+$ T_{reg} precursor cells and T_{regs}, or AhR translocation to the nucleus. While an increased (trans)differentiation is desirable in metabolic and autoimmune disease, the opposite would be beneficial in cancer diseases. As cell differentiation processes take some time, it may be reasonable to choose longer-term recovery measurement time points for blood or tissue sampling. Increases in T_{reg} counts immediately after exercise may result from effects other than true differentiation events of naïve $CD4^+$ T cells (e.g. demargination or transmigration).

As noted by Pallotta (98), TGF-β is critical in establishing a regulatory phenotype in plasmacytoid dendritic cells which in turn maintains the function of T_{regs} through constant KYN production. Thus, T_{regs} may sustain their activity by a self-reinforcing process through TGF-β production and signaling to dendritic cells. In this regard, exercise-induced increases in serum/tissue TGF-β or $CD4^+TGF\text{-}\beta^+$ regulatory T helper 3 cells (Th3) may represent important additional contributors to this signaling cascade. However, Rehm et al. (111) found an increase in $TGF\text{-}\beta^+$ producing Th3 cells, but no changes in T_{regs} one week after a marathon. Moreover, resting levels of Th3 are increased in marathoners compared to healthy controls, while T_{regs} are decreased (110). Therefore, future in vitro approaches that ensure cell-dependent or cell-independent interactions between T_{regs}, plasmacytoid dendritic cells, and Th3 cells may give insights into possible mechanistic interrelationships.

The other mechanism for T_{reg} induction that has not been addressed in original research yet, but was already discussed elsewhere (31), is the role of exercise-induced alterations in cellular metabolic pathways that may be important for T_{reg} functioning. The metabolic machinery in T_{regs} differs significantly from conventional effector T cells (42) in relying mainly on oxidative phosphorylation. It was recently shown that the mitochondrial complex III is necessary to maintain suppressive function in T_{regs} (142). It is suggested that exercise activates the histone deacetylase SIRT1 and SIRT3, which in turn promotes mitochondrial biogenesis and increases mitochondrial oxidative capacity (136). SIRT1 partly contributes to mitochondriogenesis through PGC-1α-dependent mechanisms. Further, the deletion of SIRT3 and PGC-1α leads to the loss of T_{reg} suppressive function both in vitro and in vivo (7). Interestingly, KYNA enhances PGC-1α expression and cellular respiration in adipocytes (3). These findings point to exercise-induced immunometabolic adaptations in T_{regs}. Thus, it remains to be elucidated if exercise can modulate T_{reg} differentiation and functioning through these pathways.

Tissue-specificity

In the human studies only circulating T_{regs} have been investigated in response to exercise interventions. However, T_{reg} cells are known to display tissue-specific heterogeneity. According to recent single-cell RNA and genome-wide sequencing studies, significant tissue-specific T_{reg} signatures were identified as "tissular programs" and suggest continuum-like differentiation states, adding even more complexity to T_{reg} phenotyping (26, 89). Although these findings are relatively new, unique T_{reg} phenotypes in tumor tissue (75) have been identified ear-

lier. A recent meta-analysis suggested that regular exercise may slow down tumor growth in rodents (39). Targeting T_{reg} functionality or eradicating T_{reg} subpopulation, i.e. terminally differentiated effector T_{regs}, is a hot topic in current cancer immunotherapy (130). Of the reviewed studies, Hagar et al. (47), Zhang et al. (154), and McClellan et al. (84) revealed a reduction in tumor growth and a decrease in T_{regs} or T_{reg}-related outcomes in tumor tissue. Identifying molecular pathways that may underly the proposed exercise-induced anti-tumor effects of T_{regs} should be the focus of future preclinical models. Due to heterogeneity in the validity of different tumor mouse models (44), subsequent validation in human cancer cohorts is of high clinical importance. Further, it would be interesting to understand how exercise influences the metabolism in the tumor microenvironment, as the tumor cell-induced microenvironment is implicated in T_{reg} differentiation and, therefore, increased immunosuppression (23).

It widely accepted that accumulation of excessive adipose tissue significantly contributes to disturbed immune homeostasis and is associated with decreased local T_{reg} counts and function (13, 50). Despite the known role of exercise-induced improvements in murine adipose tissue inflammation due to a suggested macrophage class switch from inflammatory M1 to anti-inflammatory M2 (61) and a proposed increase in local T_{regs} (68), no study to date investigated alterations in T_{reg} number/proportion or function in adipose tissue. Given the results of Dorneles et al. (29) and dos Santos et al. (32, 91) regarding reduced circulating T_{regs} in obesity and the positive association between CRF and circulating T_{regs} (29, 30, 64, 143), it remains elusive why no research made use of already existing improvements in techniques to gather human abdominal subcutaneous adipose tissue (5) to close this knowledge gap. From a mechanistic point of view, KYNA could be a molecule of interest being upregulated in response to exercise. It is involved in the expression of M2 macrophage and T_{reg} genes in murine adipose tissue (3). The adipokine leptin, which is mainly produced by adipocytes, may represent another target molecule as it can decrease T_{reg} differentiation while simultaneously increasing T helper 1 cell responses (24, 82).

Post-exercise T_{reg} kinetics – apoptosis and redistribution

Some studies explain exercise-induced reductions in circulating T_{regs} (21, 59) with increased apoptosis. This mechanism has been observed in other human and animal immune cells. It is likely mediated by the training status as well as exercise intensity (71). However, despite remarkable similarities of expressed genes responsible for apoptosis between mice and humans, the human regulatory machine is more complex making a flawless translation of findings in animals difficult (109). Krüger et al. (69) found that acute continuous moderate exercise, but not HIIT, provoked a significant increase in apoptotic T_{regs} three hours post-exercise compared to baseline levels. This finding was explained by an association between circulating markers of oxidative stress and apoptotic T_{regs}. However, this association is partly contradictory to the concept of an intensity-dependent increase of oxidative stress (106) which would suggest a higher number of apoptotic T_{regs} in response to HIIT. Interestingly, no significant group difference in oxidative stress markers between HIIT and moderate training groups were observed. A hypothesis attributes the beneficial hormetic-like effect of regular exercise to oxidative stress-associated damage by upregulation of cellular antioxidants and damage repair enzymes after acute exercise (105). This suggests that the antioxidant capacity from the untrained participants of Krüger et al.´s study (69) may be lower compared to that of the trained marathoners and rowers in the studies of Clifford et al. (21) and Juszkiewicz et al. (59). Thus, further complexity is added to an adequate comparison of study results. In regards to apoptosis sensitivity, it has been shown earlier that highly differentiated T cells are less sensitive to oxidative stress-induced apoptosis compared to low differentiated T cells (120, 134). However, this may not account for T_{regs}. When blood-isolated naïve $CD45RA^+$ and $CD45RA^-$ memory T_{regs} from healthy humans were exposed to pro-oxidants, naïve T_{regs} showed higher resistance to both cell death and suppressive decline compared to memory T_{regs} (92). Therefore, further research is warranted to test exercise-induced changes in apoptosis sensitivity in T_{reg} subpopulations as well as intracellular levels of antioxidants and damage repair enzymes.

Clifford et al. (21) and Juszkiewicz et al. (59) also discussed T_{reg} redistribution to peripheral tissues as an underlying mechanism for decreased circulating concentrations following exercise. The exercise-induced mobilization and subsequent migration of leucocytes (2), i.e. in T cells (16, 70, 125) and NK cells (100), is an established concept in exercise immunology. Krüger et al. (69) found an association between norepinephrine plasma levels and mobilized T_{regs} which partly confirms the catecholamine-dependent mechanism of T cell mobilization (27, 70).

Mobilized leukocytes show higher expression of integrins, adhesion molecules, and a range of chemokine receptors necessary for effective cell wall attachment and tissue transmigration. Only one of the reviewed studies considered phenotyping of $CD4^+Foxp3^+$ T_{regs} for adhesion molecules (CD44, CD62L) and chemokine receptors (CXCR4, CCR9) (137). A current review highlights the fundamental migratory role of T_{regs} for controlling local inflammation at the "sites of need" (20). Therefore, the precise characterization of human and animal T_{regs} by staining for selected integrins, adhesion molecules, and/or chemokine receptors is mandatory in future exercise immunology research. Reoccurring evidence from various disciplines reveals different expression patterns between animal and human immune cell homing factors. This fact needs to be carefully considered when translating results from the preclinical into the clinical setting (20, 33, 86) and when planning future research.

Taken together, it cannot be ruled out that both T_{reg} apoptosis and T_{reg} redistribution may occur at the same time. As with other immune cells, it is possible that unfavorable T_{reg} subsets, depending on the (disease) context, are deleted by apoptosis, while favorable T_{regs} migrate into target tissues to balance inflammation and to guarantee immune homeostasis (see figure 3). Despite species-specific differences in the $CD4^+$ T cell compartment regarding longevity and expression of adhesion molecules and chemokine receptors, measuring acute exercise effects in animal models will be mandatory to elucidate migration pattern of T_{regs} to different tissues.

T_{reg} functionality and infection susceptibility

Referring to the aforementioned role of catecholaminergic signaling in T cell mobilization after exercise, the results of Dugger et al. (37) may give mechanistic insights into exercise-induced catecholamine-dependent changes in T_{reg} functionality. By using a mouse model of allergic asthma and $\beta2AR^{-/-}$ knockout T_{regs}, Dugger et al. (37) found that reduced intracellular cAMP lev-

els in the T_{reg} $\beta2AR^{-/-}$ variants were associated with a decreased contact-dependent suppressive function after chronic exercise when compared to exercise-trained mice that harbor wild type $\beta2AR^{+/+}$ T_{regs}. By using this approach, they translated similar findings from Bopp et al. (12) into the exercise immunology context. The catecholamine-dependent increase in T_{reg} functionality was confirmed by increased cAMP levels after pharmacological β2AR agonist application. As all these observations were made in both healthy and asthmatic mice, a universal catecholamine-dependent mechanism can be assumed. More importantly, the cAMP-dependent functionality was confirmed in human T_{regs} when their function was assessed in vitro and in a humanized mouse model in vivo (62). For future research, it would be interesting to examine how T_{reg} phenotypes, ranging from naïve to terminally differentiated, differ in β2AR expression, catecholamine responsiveness, and intracellular cAMP levels when analyzed in physically active/inactive humans or in response to exercise.

Some studies propose a higher susceptibility to upper respiratory tract infection and recurrent infections in athletes due to exercise-related increases in regulatory T cell counts or function (45, 98, 126). This could at least partly be explained by the catecholaminergic mechanism delineated by Dugger et al. (37), as the increase in catecholamine levels during exercise seems to be higher in in highly trained compared to untrained subjects, also known as the "sports adrenal medulla" (156). Factors such as age, exercise type, duration, and intensity contribute to the catecholamine response (156). These factors should be taken into account when designing studies, conducting statistical analyses, or interpreting results. Moreover, some studies found positive correlations between CRF and T_{reg} counts or proportions (29, 30, 64, 143). In contrast, the serum of athletes taken immediately after a marathon was shown to exert an increased suppressive effect on T_{reg} proliferation (102). In this regard, it would be interesting to compare cAMP levels and catecholamine-responsiveness in athletes and non-athletes depending on their CRF and physical activity levels.

However, the immunosuppressive effect of both acute and long-lasting intense exercise is still a matter of debate and was discussed extensively in the last issue of this journal (124). There is a clear indication to conduct further studies that focus on exercise-induced changes in T_{reg} function in conjunction with clinical outcomes of immunosuppression. Lowder et al. (79) and Fernandes et al. (40) found increased T_{reg} counts and proportions in lung tissue from asthmatic mice after chronic exercise. Interestingly, T_{reg} suppressive capacity in lung tissue after exercise was elevated in both asthmatic and healthy mice, although no changes in T_{reg} counts and proportions were observed in healthy mice. Whether the higher anti-inflammatory or the immunosuppressive capacity has context-dependent advantageous or disadvantageous implications remains to be elucidated. However, as mice harbor significant bronchus-associated lymphoid tissue, whereas humans do not (97), the transferability of results may be difficult when appropriate mouse models, e.g. mice with humanized-lungs, are not applied. Whether such models are compatible with studies in the sports medicine field, without exerting significant confounding effects on exercise physiology, remains to be answered.

Role of exercise on T_{regs} in the elderly

There is an increasing interest in the role of exercise in immunosenescence (35, 132). An existing concept describes an exercise-induced decline in senescent or exhausted T cells, e.g. via apoptosis, to "make space" for newly built, naïve T cells, thereby boosting immunocompetence (123). However, most of the research focused on $CD8^+$ T cells. Concerning T_{regs}, mainly apoptosis-resistant T_{reg} subsets accumulate in the elderly which is associated with a higher risk of infections through increased immunosuppression (55, 72). This may be due to an age-dependent decrease in the expression of the pro-apoptotic molecule Bim in T_{regs} which was found in mice (19). Although it is known that the proportion of naïve $CD45RA^+$ T_{regs} decreases, while memory $CD45RO^+$ T_{regs} increase with age (11), there is evidence that $CD45RA^+$ T_{regs} are highly apoptosis-resistant compared to their memory counterpart (92). This may challenge the idea of replacing memory T cells by naïve cells to counteract immunosenescence in the context of T_{regs}. Increases in $CD25^+Foxp3^{dim}CD45RA^+$ naïve T_{reg} counts and decreases in $CD25^+Foxp3^{high}CD45RA^-$ memory T_{reg} counts after a strenuous multi-day march in octogenarians (81.3 ± 1.9 years) (135) indicates the mobilization of more apoptosis-resistant cells. As shown by Duggal et al. (36), a large cohort of master cyclists (55–79 years) has decreased proportions of $CD4^+CD25^{high}Foxp3^+$ T_{regs} compared to inactive age-matched controls, whereas these levels do not differ from inactive young adults. That may support an exercise-induced delay of immunosenescence. However, since only T_{reg} proportions were considered without measuring cell counts, some of the differences between the young inactive and old active subjects may be misleading as lymphocyte numbers fall with age. Further, low and highly differentiated T_{regs} were not specified. In response to acute exhaustive exercise (88), master athletes of a younger age (53.2 ± 9.08 years) showed no alterations in counts and proportions of T_{reg} subsets (low/highly differentiated $CD45RA^{+/-}$, terminally differentiated $KLRG1^+$). However, no study assessed T_{reg} functionality. As this parameter is the most reliable to verify changes in (age-related) immunosuppression, contact-dependent and/or contact-independent suppressive capacity should be addressed in future exercise studies. Additionally, exercise-induced effects on apoptosis sensitivity and antioxidant capacity in high and low differentiated T_{reg} subsets should be measured in elderly people.

In the literature, it is suggested that the ratio of peripherally induced T_{regs} (pT_{regs}) to thymus-derived T_{regs} (tT_{regs}) decreases with age due to thymic involution and a concomitant decline in RTEs as the main precursors of pT_{regs} (22). This is a proposed mechanism for the increased immunosuppressive state in the elderly. Interestingly, the cell counts of $CD31^+$ RTEs increased significantly in octogenarians after a strenuous multi-day march (135). Moreover, higher proportions of $PTK7^+CD45RA^+$ RTEs were observed in master cyclists compared to age-matched inactive controls with the same levels seen in the younger group (36). However, there is no valid method to date to truly differentiate between pT_{regs} and tT_{regs}, since the best-known proposed marker of tT_{regs}, the transcription factor Helios, is still controversial (43, 131). Research during the last decade revealed important epigenetic regulatory mechanisms within the Foxp3 locus, i.e. the promotor and three conserved non-coding sequences, that are necessary for stable Foxp3 expression. The first sequence is needed for pT_{reg} generation, but is dispensable for tT_{reg} generation (52). Therefore, elucidating what determinants of exercise (modality, duration, frequency) induce histone modification and change gene expression of transcription factors and their binding to the first non-coding sequence of the Foxp3 locus could give future implications for increasing peripherally induced T_{regs} while simultaneously decreasing thymus-derived T_{regs} in the elderly.

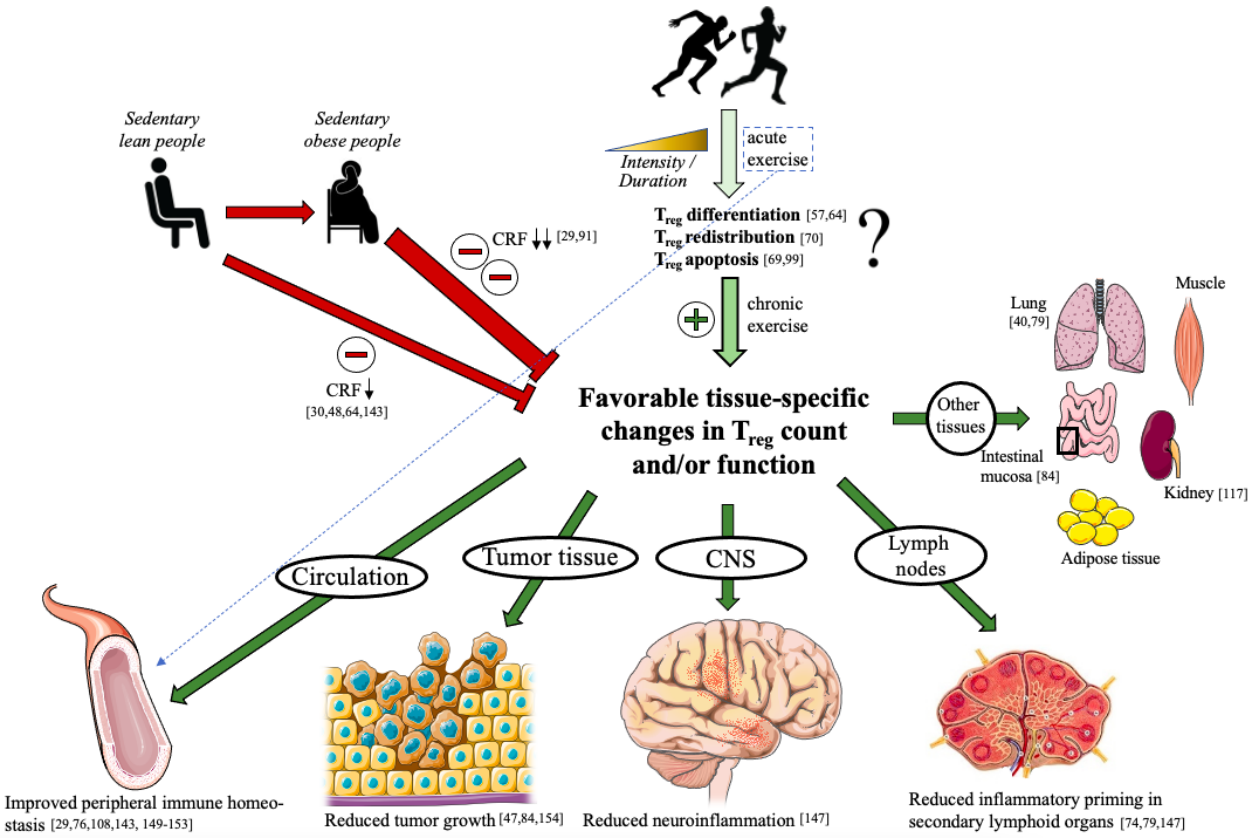

Figure 3: Schematic illustration of proposed exercise-induced effects on regulatory T cells and clinical implications. Acute exercise may lead to T_{reg} differentiation or redistribution and apoptosis of T_{reg} subsets in an intensity- and duration-dependent manner. Repetitive acute bouts over several months, namely chronic exercise, may consequently lead to favorable tissue-specific changes in T_{reg} count and/or function, depending on the (disease) context. This results in reduced tumor growth and metastatic spread, decreased neuroinflammation and loss of neural tissue, reduced inflammatory priming and generation of inflammatory (autoreactive) T cells, and improved peripheral immune homeostasis. T_{reg} count and functionality in other tissues like the lung, muscle, kidney, intestinal mucosa, and adipose tissue may also be beneficially influenced by chronic exercise. A decline in cardiorespiratory fitness due to inactivity has detrimental effects on immunity and favor T_{reg} dysregulation. The establishment of an obese state with concomitant decreases in CRF further diminishes immune regulation. Of note, the effects of acute exercise on T_{regs} have only been examined in the human blood compartment (blue arrow). Corresponding references are provided in brackets.
CNS: central nervous system; CRF: cardiorespiratory fitness; T_{reg}: regulatory T cell

Recommendations for future research

The single sections within the discussion integrated findings of exercise-induced changes in T_{reg} measures into current frameworks of exercise immunology and emphasized existing knowledge gaps. Several key elements should be considered to improve quality, validity, and interpretation of results while simultaneously reducing heterogeneity. Thus, to promote future research in this field, it is recommended that researchers should

- consider T_{reg} measures as primary outcomes with powered sample sizes to draw clearer conclusions on exercise-induced effects. To date, T_{reg} measures often represent exploratory endpoints.
- adhere to the panel proposed by Santegoets et al. (119) for proper T_{reg} characterization in humans. Basal phenotyping should include CD4, CD25, and CD127 to isolate T_{regs} for functional assays, and CD4, CD25, and Foxp3 to measure T_{reg} counts or calculate T_{reg} proportions. The same strategy accounts for rodent T_{regs} (112). It is further suggested to use CD45RA, CD45RO, and CD95 for discriminating between human naïve and highly differentiated/memory T_{regs} (93). For defining mouse memory T_{regs}, high expression of CD27 and CD44 is proposed (114). Surface CD39 and intracellular CTLA-4 expression can be used to identify highly suppressive T_{regs} in humans, while in mice, CD39 and CD73 should be used (63). CD31 measurement is proposed to characterize human RTE T_{regs} (46).
- stain T_{regs} for integrins, adhesion molecules and/or chemokine receptors. Naïve T_{regs} express CD62L (for rolling) and both CCR7 and LFA-1 (for adhesion), while PSGL-1 and CD44 are rather expressed on activated T_{regs} (20). The expression of these molecules highly depends on the disease context and target tissue of which the T_{regs} are attracted by. For example, chemokine receptors that are expressed on T_{regs} and are implicated in migration to tumor sites are CCR4, CCR7, CCR8, CXCR4, and CXCR5 (53, 155). In this context, cell migration and invasion assays of T_{regs} represent promising approaches to reveal tissue-specific properties of adhesion and migration (58).
- calculate and report the delta of pre and post values regarding T_{reg} viability when an acute exercise protocol was applied. This may be valuable to gain insights into exercise-induced T_{reg} fate and to not misinterpret an increase in cell counts. T_{regs} should be further stained for apoptosis markers (e.g. Annexin V, caspase 3 or 9, CD95) to identi-

fy viable but non-functional cells (99).

- use functional assays to quantify T_{reg} suppressive capacity as a main outcome. To avoid heterogeneity, researchers are referred to a current review that addresses important issues (e.g. choice of responder T cells, monitoring suppression of proliferation) when conducting an ex vivo approach (54). It is recommended to do functional testing with freshly isolated T_{regs}. When using cryopreserved T_{regs}, appropriate cryopreservation-thawing techniques should be applied to prevent decreases in viable T_{regs} (4).
- calculate T_{reg} proportions relative to the CD4 population instead of the lymphocyte or leukocyte compartment. To overcome the wide heterogeneity regarding T_{reg} count units, it may be reasonable to find a consensus on one unit for flow cytometry and one unit for immunohistochemical studies that should be used throughout future studies. Increasing homogeneity in presentation of T_{reg} proportion and counts will allow meta-analysis approaches.
- conduct more acute exercise studies in humans that include blood sampling at several measurement time points, i.e. immediately (within one minute), ten minutes, one hour, three hours, and 24 hours post-exercise. This will give more detailed information on exercise-induced alterations of circulating T_{regs}.
- conduct acute exercise studies in animals to examine acute effects on tissue-specific or tissue-infiltrated T_{regs}.
- conduct more chronic studies in humans, especially in diseased populations, with at least ten weeks of exercise to guarantee chronic adaptions in the T_{reg} compartment. Further, varying exercise modalities (upper body vs. lower body or combined, resistance vs. endurance or combined exercise) and intensities (moderate vs. intense or alternating exercise) should be applied to increase knowledge of intensity- and modality-specific effects on T_{reg} measures.
- should wait at least 24 hours to take blood samples after chronic interventions, since acute effects on cytokines and cellular immune components need approximately 24 hours to disappear. Further, reporting on measurement time points should be as detailed as possible. Reporting like "blood samples were collected at least 24/48/72 hours after the last training session" hampers interpretation and reproducibility.
- do consequent and precise reporting on methods and study design, e.g. according to the TESTEX (127) and OHAT (157) scales, to reduce the risk of bias and improve study quality. In this context, reporting of the exercise prescription components of frequency, intensity, time, and type (FITT) in chronic human studies is of special interest as they are crucial for optimizing exercise interventions and are suspected to influence exercise adherence (15, 94).
- make use of existing methods in molecular immunology for in-depth analysis (e.g. chromatin immunoprecipitation, single cell sequencing, -omics approaches) to examine exercise-induced changes in T_{reg} cellular metabolism or epigenetic alterations. This accounts especially for animal models when analyses are done in tissue-specific T_{regs} such as CNS, peripheral organs, and tumor microenvironment.

Limitations

Due to the holistic approach of this review covering all research in the exercise-T_{reg} context, some limitations should be mentioned. The review considered the inclusion of all T_{reg} phenotypes, i.e. those $CD4^+$ T cells only stained for $CD25^+$ or $Foxp3^+$ that is today known as being insufficient for T_{reg} characterization (112). Further, studies that link gene expression of Foxp3 in any immune cell compartment to T_{regs} were included, while only Foxp3 gene expression in the $CD4^+CD25^+(CD127^-)$ should be considered appropriate. Another limitation may be the inclusion of studies with low quality or a high risk of bias confining the validity of results. In this regard, no assessment of acute studies was done, as there is no valid tool to be used to calculate study quality or risk of bias.

CONCLUSION

Regulatory $CD4^+$ T cells represent the main and best studied regulatory component within the adaptive immune system and are increasingly appreciated as a cornerstone for immune therapy approaches in cancer and autoimmune diseases. From the included studies, only a limited number examined exercise-induced effects on T_{reg} functionality which, however, is of high clinical significance and should therefore be addressed by future studies. A disease-specific beneficial effect of chronic exercise on T_{reg} measures can be stated from animal models, that is an increase in T_{reg} levels during autoimmune conditions or chronic inflammation and a decrease of T_{reg} levels in cancer. As T_{reg} phenotyping in the included animal studies is largely insufficient, these findings should be interpreted with caution and be validated with appropriate staining procedures. Due to the relatively high risk of bias, drawbacks in study design, and the large heterogeneity in human and animal studies (i.e. population, outcome measure specification, exercise protocol), no clear overall conclusions can be drawn. Unraveling the impact of exercise on systemic and tissue-specific T_{reg} functionality and phenotypic changes in future studies is highly warranted. It should further be taken into account that regulatory $CD4^+$ T cells and their subpopulations are not the only cells within the immune system that possess immunosuppressive functions. Therefore, it might be reasonable to include other cell types with suppressive characteristics when analyzing exercise-induced effects on the immune system to view the whole (complex) picture instead of considering only one piece of the puzzle. Emerging cell types of interest are the Myeloid-Derived Suppressor Cells (96), different subtypes of $CD8^+$ regulatory T cells (41) and regulatory B cells (115). Methodological recommendations and research questions that are outlined in this review should be considered in the future when studying healthy and diseased humans or respective animal models. However, the results of animal studies should always be interpreted with caution as "mice are not men" in regards to immunology.

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SUPPLEMENTARY MATERIAL

Table S1: Rating of human chronic exercise studies according to (127).

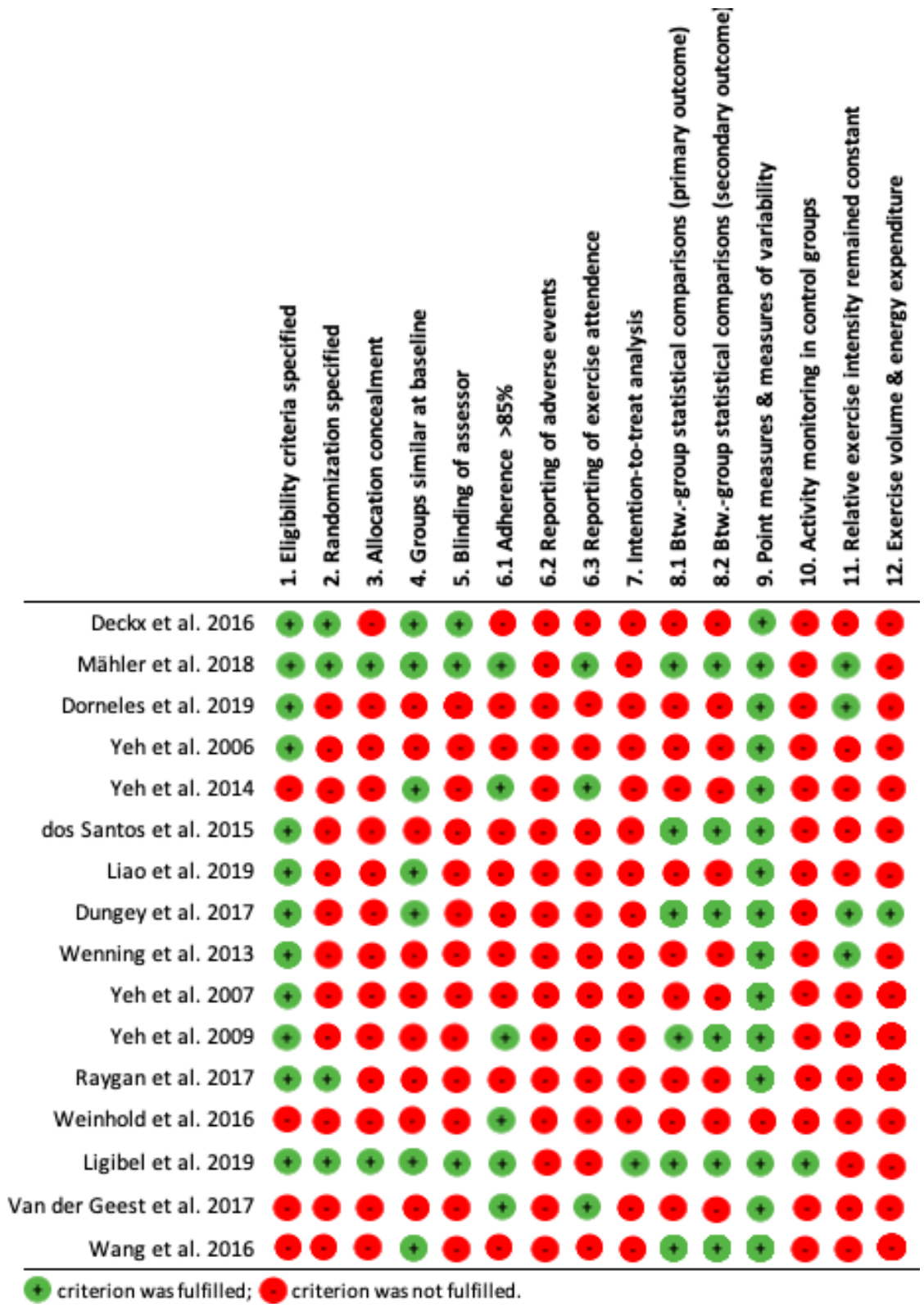

Table S2: Rating of animal studies according to (157).

		Zhang et al. 2016	Bianco et al. 2019	dos Santos et al. 2019	Hagar et al. 2019	Abdalla et al. 2013	McClellan et al. 2014	Miyagi et al. 2018	Chen et al. 2018	Jakic et al. 2019	Barhoumi et al. 2014	Le Garf et al. 2019	Yin et al. 2015	Song et al. 2018	Lowder et al. 2010	Dugger et al. 2018	Fernandes et al. 2019	Rael et al. 2019	Uchiyama et al. 2014	Souza et al. 2017	Xie et al. 2019	Bernardes et al. 2016	Walker et al. 2018	Wang et al. 2011
Selection bias?	1 Was the administered dose of exposure level adequately randomized?	-	-	+	+	-	+	-	+	-	-	+	-	-	+	+	-	-	+	+	+	+	-	+
	2 Was allocation to study groups adequately concealed?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Performance bias?	3 Were experimental conditions identical across study groups?	-	+	+	+	+	+	+	-	+	-	+	-	-	-	+	-	-	-	+	-	-	-	-
	4 Were the research personnelblinded to the study group?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Attrition/exclusion bias?	5 Were outcome data complete without attrition or exclusion from analysis?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Detection bias?	6 Can we be confident in the exposure characterization?	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
	7 Can we be confident in the outcome assessment?	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Selective reporting bias?	8 Were all measured outcomes reported?	++	+	++	++	++	+	+	+	++	++	++	+	+	+	++	++	+	+	+	+	++	++	+
Other bias	9 Were statistical methods appropriate?	Y	Y	Y	N	Y	N	N	Y	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N
	10 Did researchers adhere to the study protocol?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	11 Did the study design or analysis account for important confounding variables in experimental studies?	Y	Y	Y	Y	Y	*NA*	*NA*	*NA*	*NA*	*NA*	Y	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*

++ definitely low risk of bias (dark green); + probably low risk of bias (light green); – probably high risk of bias (light red); – – definitely high risk of bias (dark red).

NA: Not Applied