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High-intensity interval training in allogeneic adoptive T-cell immunotherapy – a big HIT?

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Abstract

Background: Adoptive transfer of virus-specific T cells (VSTs) represents a prophylactic and curative approach for opportunistic viral infections and reactivations after transplantation. However, inadequate frequencies of circulating memory VSTs in the T-cell donor's peripheral blood often result in insufficient enrichment efficiency and purity of the final T-cell product, limiting the effectiveness of this approach.

Methods: This pilot study was designed as a cross-over trial and compared the effect of a single bout (30 min) of high-intensity interval training (HIT) with that of 30 min of continuous exercise (CONT) on the frequency and function of circulating donor VSTs. To this end, we used established immunoassays to examine the donors' cellular immune status, in particular, with respect to the frequency and specific characteristics of VSTs restricted against Cytomegalovirus (CMV)-, Epstein–Barr-Virus (EBV)- and Adenovirus (AdV)-derived antigens. T-cell function, phenotype, activation and proliferation were examined at different time points before and after exercise to identify the most suitable time for T-cell donation. The clinical applicability was determined by small-scale T-cell enrichment using interferon- (IFN-) γ cytokine secretion assay and virus-derived overlapping peptide pools.

Results: HIT proved to be the most effective exercise program with up to fivefold higher VST response. In general, donors with a moderate fitness level had higher starting and post-exercise frequencies of VSTs than highly fit donors, who showed significantly lower post-exercise increases in VST frequencies. Both exercise programs boosted the number of VSTs against less immunodominant antigens, specifically CMV (IE-1), EBV (EBNA-1) and AdV (Hexon, Penton), compared to VSTs against immunodominant antigens with higher memory T-cell frequencies.

Conclusion: This study demonstrates that exercise before T-cell donation has a beneficial effect on the donor's cellular immunity with respect to the proportion of circulating functionally active VSTs. We conclude that a single bout of HIT exercise 24 h before T-cell donation can significantly improve manufacturing of clinically applicable VSTs. This simple and economical adjuvant treatment proved to be especially efficient in enhancing virus-specific memory T cells with low precursor frequencies.

Keywords: Virus-specific T cells, T-cell manufacturing, Adoptive T-cell immunotherapy, High-intensity interval training

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Background

Latent herpes viruses such as Cytomegalovirus (CMV) and Epstein–Barr virus (EBV) as well as lytic viruses such as Adenovirus (AdV) remain an important cause of morbidity and mortality after hematopoietic stem cell transplantation (HSCT) and solid organ transplantation (SOT) in immunocompromised patients [1–3].

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With a median of 3 month after HSCT, 11% of all deaths are induced by viral infections with about one-third are caused mainly by CMV (up to 20%), EBV (up to 50%) and AdV (up to 75%) [4–10]. With an incidence of 6 to 28%, AdV infections are the most common infectious complications after HSCT, especially in children [2, 5] Viral complications also influence the outcome of SOT [11] and in general, the incidence varies depending on the transplanted organ, the resulting immunosuppressive treatment, the serostatus of the donor and recipient, and the type of antiviral prophylaxis [12]. Due to high immunosuppression reactivation of EBV and/or outgrowth of latently EBV-infected cells can result in the development of malignant lymphoma (post-transplant lymphoproliferative disease; PTLD), which represents the most common EBV-associated malignancy after transplantation [13].

The pharmacotherapy of viral complications is often limited by both lack of efficacy and toxic side effects. Adoptive transfer of virus-specific T cells has become an attractive option for second line treatment [5, 14–17]. It is established that the delay in the reconstitution of virus-specific $CD8^+$ and/or $CD4^+$ T-cell responses is a critical factor in viral recrudescence and viral disease and that the permanent control and elimination of a virus depends on the presence of functional virus-specific T cells (VSTs) [18, 19]. The adoptive transfer of donor-derived memory VSTs with defined specificity has developed into a prophylactic and/or curative approach for common post-HSCT and post-SOT viral infections without a significant risk of toxicity or GvHD [2, 3, 20–26]. Recently, we demonstrated the successful use of EBV-specific T cells as a consolidating treatment for a patient suffering from EBV-associated PTLD of the central nervous system [27].

Clinical-grade VSTs can be rapidly isolated directly from blood leukapheresis donations by the cytokine capture system (CliniMACS CCS) [26, 28, 29].

The major challenge is the enrichment of VSTs in sufficient quantities against pathogens with low frequencies of memory VSTs present in the circulation of peripheral blood (e.g. AdV-specific T cells). However, clinical studies showed that the adoptive transfer of even relatively low numbers of VSTs (0.4×10^3 to 8×10^4/kg CMV-VSTs, 0.2×10^3 to 6×10^4/kg EBV-VSTs, 0.3×10^3 to 3×10^4/kg AdV-VSTs) resulted in the decrease in viral load without acute toxicities or GvHD induction [2, 5, 24, 26]. Studies over the last 30 years successfully demonstrated the feasibility of the adoptive T-cell transfer with a treatment response in 74% of 246 evaluable published patients including 85% of CMV-responders, 62% of EBV-responders and 74% of AdV-responders [5, 18, 30–35]. Still, some patients do not respond to VST infusions, possibly because the infusions contain low numbers of VSTs [36].

Methods to increase the number and function of VSTs in healthy donors are an attractive way to ensure the success of adoptive T-cell therapy. As lymphocytosis occurs during and directly after exercise [37], pre-donation exercise could be a time- and cost-efficient way to increase the starting frequencies of circulating peripheral memory VSTs in the donor before apheresis [38–40]. Especially single bouts of exercise evoke increased hemodynamics and the release of catecholamines and glucocorticoids, resulting in a striking leukocytosis [37]. Simultaneously, a redistribution of effector cells between the blood compartment and the lymphoid and peripheral tissues occurs [37]. On the other hand, longer periods of exhaustive exercise can have the adverse effect and weaken immunity [37]. Recent studies have shown that a single bout of continuous exercise (CONT) enhances the quantity of VSTs when they are stimulated and expanded over 8 days [39]. In the study of Kunz et al. they clearly demonstrated that single bouts of exercise can mobilize AdV-specific T cells and increase their function [41]. There is a growing body of evidence showing that high-intensity interval training (HIT) is an effective alternative to traditional endurance training. Alongside similar or even superior effects on many health-related markers (e.g. cardiovascular risk factors), lymphocyte numbers were shown to increase directly after HIT in sedentary men [38–40, 42, 43].

The present study aimed to investigate the benefit of CONT and HIT for adoptive antiviral T-cell therapy. We compared the impact of a 30 min single bout of HIT versus 30 min of CONT on the cellular immune status of healthy donors, in particular, regarding the total number, functionality and proliferative capacity of VSTs. These parameters were analysed in blood samples collected at different time points before and after exercise to identify the time point with the most effective induction level of functional active VSTs and therefore to define the optimal time point for T-cell donation. In summary, we found that both training protocols boost the number and function of VSTs in healthy donors and that the highest response was observed in VSTs against CMV, EBV and AdV with low precursor frequencies in the peripheral blood and in donors with a moderate fitness level who performed HIT 24 h prior to donation.

Methods

Study population

After approval by the Institutional Review Board of Hannover Medical School (MHH, approval number 3366-2016), 12 healthy adult volunteers (six females and six males with a mean age of 24 years; range: 20–31 years) were recruited into this study. Their demographic characteristics are presented in Table 1. To be eligible

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Table 1 Physical and fitness characteristics of the participants

Characteristics	Group 1 (n = 6)	Group 2 (n = 6)	Total (n = 12)
Sex	3 females, 3 males	3 females, 3 males	6 females, 6 male
Age (years)	22.50 ± 2.07	25.67 ± 4.46	24.08 ± 3.70
Height (cm)	180.33 ± 10.41	175.17 ± 5.15	177.75 ± 8.28
Mass (kg)	77.78 ± 26.34	80.05 ± 13.31	78.92 ± 19.93
BMI (kg/m^2)	23.44 ± 4.82	26.04 ± 4.09	24.73 ± 4.47
Fitness level (IPAQ-score)	4 moderate, 2 high	4 moderate, 2 high	8 moderate, 4 high
Individual performance limit (W)	221.20 ± 36.27	236.50 ± 41.20	228.83 ± 37.90
Individual performance limit (W/kg)	2.97 ± 0.69	2.9 ± 0.50	2.93 ± 0.57

Each donor executed the two exercise protocols in crossover fashion. At the first visit (week 1), one group (n = 6, group 1) performed a single bout of continuous exercise (CONT) at 50% P$_{max}$ for 30 min, while the other group (n = 6, group 2) performed a single bout of high-intensity interval training (20 min of 30-s 100% P$_{max}$ intervals each followed by a 30-s pause 5 min before and after HIT exercises at 50% P$_{max}$, more precisely High Intensity High Volume Training, HIHVT). At the second visit (week 3), each group switched over to the other exercise protocol. In other words, group 1 did continuous exercise during the first visit now and did HIT exercise on the second (CONT/HIT), and group 2 vice versa (HIT/CONT)

participants had to be over 18 years old, not pregnant and without any evidence of disease. After giving their written informed consent, each participant underwent a medical check-up to exclude active infections and an electrocardiogram in rest was evaluated by an experienced cardiologist to make sure that participants could join the exhaustion test. The participants' fitness level was assessed using the International Physical Activity Questionnaire (IPAQ) [44] for classification of fitness level. All 12 donors had a moderate (n = 8) or a high (n = 4) fitness level.

Exercise protocols and blood sampling

Participants were asked to refrain from exercise 24 h prior to each laboratory visit. The individual exercise capacity as measured by maximum power output (P$_{max}$) was determined with a graded exercise test (GXT) on a cycle ergometer (Ergoline, Bitz, Germany) 4 weeks prior to testing. GXT started at 50 W, the workload increased by 16.7 W/min until physical exhaustion. Heart rate (HR) was measured continuously with a 12-channel ECG (GE Electric, Boston USA). Earlobes were hyperaemised at least for 3 min before the first sample was drawn. After 50 W, every 3 min afterwards and at exhaustion capillary blood drawn into 20 μl end-to-end capillaries was used to analyse glucose and lactate concentrations within the next 2 h. Analyses were performed with the Biosen S line analyzer (EKF Diagnostics, Barleben, Germany) including the glucose/lactate test set-up with haemolysing, Glucose/Lactate system, multi standard and Ready-Con test solutions (EKF Diagnostics). On two further occasions separated by a wash-out phase of 14 days, all 12 participants visited the Institute of Sports Medicine at MHH. They were assigned to two equally-sized groups based on the availability of the participants. Blinding was not deemed possible. Each donor executed the two

exercise protocols in crossover fashion (Table 1). At the first visit (week 1), one group (n = 6, group 1) performed a single bout of continuous exercise (CONT) at 50% P$_{max}$ for 30 min, while the other group (n = 6, group 2) performed a single bout of high-intensity interval (HIT, more precisely High Intensity High Volume Training, HIHVT) training for 20 min of 30-s 100% P$_{max}$ intervals each followed by a 30-s pause 5 min before and after HIT exercises at 50% P$_{max}$. At the second visit (week 3), each group switched over to the other exercise protocol. Heart rate was measured continuously throughout each visit with a 3-channel ECG (Ergoline, Bitz, Germany), and earlobe capillary blood samples for lactate and glucose testing were drawn before, during and after exercise. Intravenous blood samples were collected from a cubital or antecubital vein before and directly after exercise as well as 1 h and 24 h after exercise (Fig. 1). Complete blood counts and cellular immune status tests were performed immediately on whole blood samples. Peripheral blood mononuclear cells (PBMCs) were isolated by discontinuous density gradient centrifugation and examined for the specified VST characteristics using appropriate in vitro immunoassays. Representative flow cytometric plots of the cellular immune status (A), VST enrichment efficiency (B) and T-cell activation using CD69 as a specific marker (C) are visualized in Additional file 1.

Serological testing by ELISA and Western blot

All donors were pretested for CMV, EBV and AdV serostatus as described previously using commercially available IgG Western blot and IgG ELISA kits [45, 46]. Briefly, CMV and EBV serology was performed by confirmatory Western blot tests designed to quantitatively determine anti-CMV or anti-EBV IgG antibodies against major CMV and EBV proteins (recomLine CMV or EBV IgG, Mikrogen, Neuried, Germany). IgG antibodies
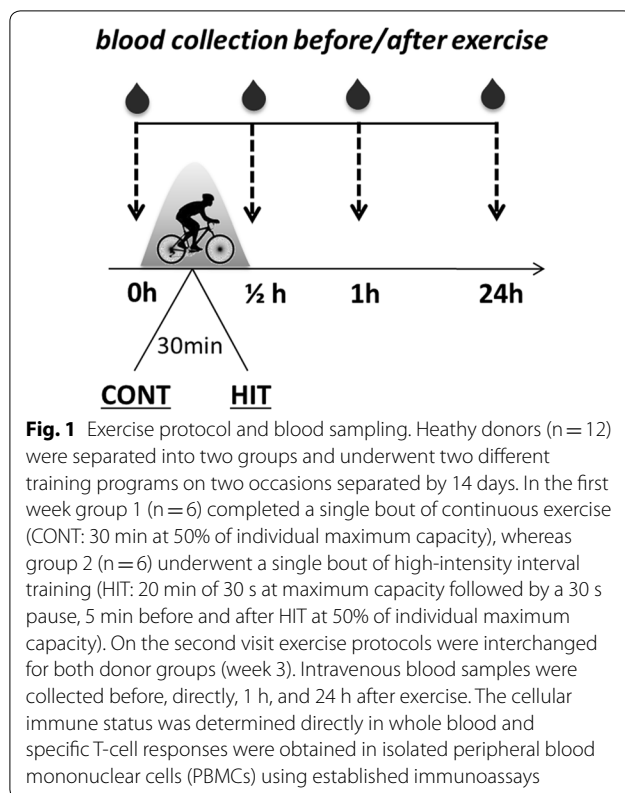
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Fig. 1 Exercise protocol and blood sampling. Heathy donors (n = 12) were separated into two groups and underwent two different training programs on two occasions separated by 14 days. In the first week group 1 (n = 6) completed a single bout of continuous exercise (CONT: 30 min at 50% of individual maximum capacity), whereas group 2 (n = 6) underwent a single bout of high-intensity interval training (HIT: 20 min of 30 s at maximum capacity followed by a 30 s pause, 5 min before and after HIT at 50% of individual maximum capacity). On the second visit exercise protocols were interchanged for both donor groups (week 3). Intravenous blood samples were collected before, directly, 1 h, and 24 h after exercise. The cellular immune status was determined directly in whole blood and specific T-cell responses were obtained in isolated peripheral blood mononuclear cells (PBMCs) using established immunoassays

against AdV were detected using the alphaWell Adenovirus IgG ELISA (Mikrogen) according to the manufacturer's instructions.

Determination of the frequencies of antiviral memory T cells by enzyme-linked immunospot assay

Detection of virus-specific IFN-γ-producing T-lymphocytes was achieved by IFN-γ EliSpot assay as previously described. Briefly, PBMCs were isolated from whole blood samples by discontinuous density gradient centrifugation, resuspended in culture medium (CM) consisting of RPMI1640 (Lonza, Vervies, Belgium) with 10% human AB serum (C.C.pro, Oberdorla, Germany) at a concentration of 1×10^7 cells/ml, seeded on 24-well plates and rested overnight. Rested PBMCs were co-cultured in anti-IFN-γ precoated EliSpot plates (Lophius Biosciences, Regensburg, Germany) for 16–18 h at a density of 2.5×10^5 cells/well with specific antigens of interest. For stimulation CMV-derived peptide pools CMV pp65 and CMV IE-1, EBV-derived peptide pools EBV EBNA1 and EBV Consensus and AdV-derived peptide pools AdV5 Hexon and AdV5 Penton were used at a final concentration of 1 μg of each peptide/ml peptide pool (Miltenyi Biotec, Bergisch Gladbach, Germany). Cells stimulated with staphylococcal enterotoxin B (1 μg/ml, SEB, Merck, Taufkirchen, Germany) served as the

positive control and PBMCs incubated in media alone as the negative control. IFN-γ secretion was detected using streptavidin–alkaline phosphatase (Mabtech Stockholm, Sweden) and revealed by 5-13 bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT Liquid Substrate, Merck). Spots were counted using AID EliSpot 8.0 on an AID iSpot spectrum reader system (both from AID, Strassberg, Germany). Findings are indicated as number of spots per well (spw), representing the number of spots in the antigen well after subtracting the respective negative control well. Results were furthermore indicates as spw/1000 CD3+ T cells.

IFN-γ Cytokine Secretion Assay

The IFN-γ Cytokine Secretion Assays (CSA, Miltenyi Biotec) was performed as previously described [47]. After overnight resting, 1×10^7 isolated PBMCs were stimulated with the CMV peptide pools CMV pp65 or CMV IE1, EBV peptide pools EBV EBNA1 or EBV Consensus, and AdV peptide pools AdV5 Hexon or AdV5 Penton, respectively, at a final concentration of 1 μg of each peptide/ml peptide pool. Unstimulated PBMCs served as the negative control. Activated IFN-γ-secreting T cells were specifically captured during the magnetic cell sorting enrichment process by using anti-IFN-γ-phycoerythrin (PE) antibodies and paramagnetic anti-PE microbeads. Aliquots of the respective cell fractions collected before and after enrichment were used for detailed analysis of IFN-γ+ T-cell subsets by multicolour flow cytometry. The distribution of viable and dead cells in these fractions was analysed by 7AAD (7-amino-actinomycin D) staining (BD Biosciences, Heidelberg, Germany). The percentage of viable IFN-γ+ cells was determined by staining the cells with anti-CD45-allophycocyanin plus cyanin-7 (APC/Cy7), anti-CD3-fluorescein isothiocyanate (FITC), anti-CD8-APC, and anti-CD4-Alexa-Fluor 700) monoclonal antibodies (mAbs, all from BD Biosciences). At least 10,000 events were acquired in the viable CD45+ leukocyte gate for each analysis (FACSCanto10c, BD Biosciences). CD3+/IFN-γ+, CD8+/IFN-γ+ and CD4+/IFN-γ+ T-cell populations were gated based on the scatter properties of viable 7AAD−/CD45+/CD3+ T lymphocytes. Results are indicated as frequencies (in %) of IFN-γ+ T cells after subtracting the respective negative control.

Detection of the cellular immune status by flow cytometry

All flow cytometric analyses were performed using the FACSCanto 10c system (BD Biosciences) and BD FACS-Diva Software version 8.0.1. The cellular immune status was determined by using specific markers for monocytes, T cells, B cells and natural killer (NK) cells. Briefly, whole blood samples were analysed on a single-cell platform

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using TruCount tubes (BD Biosciences). Absolute counts of the investigated leukocyte subsets were calculated according to the manufacturer's instructions. Cells were stained for 20 min at room temperature using anti-CD45 APC-H7, anti-CD3 FITC, CD8 APC, anti-CD4 peridinin chlorophyll protein (PerCP), anti-CD19 PE-Cy7, anti-CD56 PE and anti-CD14 BV510 mAbs (all from BD Biosciences) before the lysis of erythrocytes using $1 \times$ BD FACS Lysing Solution according to the manufacturer's instructions (BD Biosciences). The number of spots per 1000 CD3$^+$ T cells, as determined by IFN-γ EliSpot assay, was calculated based on the CD3 frequencies determined by staining PBMCs with anti-CD45 APC-H7, anti-CD3 FITC, anti-CD8 APC and anti-CD4 PerCP).

Real-time PCR detection of HSP70, Ki67, IFN-γ, and granzyme B expression measurement of telomere length

The mRNA levels of heat shock protein 70 (HSP70), Ki67, IFN-γ, and granzyme B were analysed as previously described [48]. Briefly, total cellular RNA was isolated (RNeasy Mini Kit; Qiagen, Hilden, Germany), and cDNA was amplified using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Darmstadt, Germany). Quantification of HSP70, Ki67, IFN-γ, and granzyme B mRNA levels was achieved by using inventoried mixes (Applied Biosystems). TaqMan Gene Expression Master Mix (Applied Biosystems) was used for amplification. The constitutively expressed glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene served as the reference gene.

As an indicator of telomere length, p16 expression was analysed. Quantitative PCR of p16INK4a was performed using the following intron-spanning primers and probes: p16INK4a forward 5′-GGGCACTGCTGGAAGCC-3′, reverse 5′-AACGTTGCCCATCATCATC-3′, and probe 5′-CCGAACTCTTTCGGTCGTA-3′. For analysis of telomere length, genomic DNA was first extracted from isolated PBMCs using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). DNA quantification was then performed on an Infinite M200 Pro microplate reader (Tecan, Maennedorf, Switzerland) using SYBR®Green I Dye (Invitrogen, Carlsbad, CA, USA). Telomere length was calculated as abundance of telomeric template versus a single copy gene (36B4) by quantitative real-time PCR (qPCR) on a 7900HT Fast Real-Time PCR System (Applied Biosystems, Darmstadt, Germany) as previously described [49, 50]. Results are expressed as the ratio of the telomere repeat copy number to the single gene copy number (T/S). All measurements were performed in quadruplicate, and all samples were measured on a single plate to reduce inter-assay variability.

Quantification of the total cellular cholesterol level of cytotoxic CD8$^+$ T cells

CD8$^+$ T cells were isolated from blood samples collected at different time points directly before and after exercise (Whole Blood CD8 MicroBeads, Miltenyi Biotec) for quantification of total cellular cholesterol. The cells were fixed with 0.1% glutaraldehyde (Merck, Taufkirchen, Germany) and treated with 2 U/ml cholesterol oxidase (ThermoFisher) for 15 min to oxidize the plasma membrane cholesterol. After extracting intracellular cholesterol with methanol/chloroform (vol/vol, 1:2), total cellular cholesterol was quantified using the Amplex Red Cholesterol Assay Kit (ThermoFisher) according to the manufacturer's instructions. Plasma membrane cholesterol levels were calculated by subtracting intracellular cholesterol from total cellular cholesterol. The purity of isolated CD8$^+$ T cells was assessed by flow cytometry after staining the cells with anti-CD8 APC mAb (BD Biosciences). At least 10,000 events were acquired in the CD8$^+$ T-cell gate. The percentage of activated CD8$^+$ T cells was determined by staining the cells with anti-CD3 PerCP, anti-CD8 APC, and anti-CD69 FITC mAbs (all from BD Biosciences). At least 20,000 events were acquired in the CD3$^+$ T-cell gate for each analysis, while CD69$^+$ T cells were gated on the scatter properties of CD3$^+$/CD8$^+$ T lymphocytes.

Statistical analysis

This pilot study was carried out as a non-randomized two period two sequences cross-over trial. We did not adjust for multiplicity due to the exploratory nature of this trial and hence, our results are not to be interpreted in a confirmatory sense.

Statistical analysis was performed using the Prism v5.02 software (GraphPad, San Diego, California, USA) and SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The results are displayed as mean ± standard deviation (SD). Data were analysed using the Wilcoxon test to compare each post training measurement with the pre-training measurement separately. Significance levels were calculated and expressed as p-values (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). In addition, we carried out calculations to compare the two training methods with the two sample t approach adjusting for period as described in chapter 3 of [51].

Results

Exercise induced lymphocytosis

All donors successfully completed the two 30 min exercise sessions. The effects of both protocols on the standard complete blood count (CBC) and cellular immune status were investigated with respect

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Table 2 Blood glucose, blood lactate, and heart rate in response to exercise

Parameter	Program	Before exercise	During exercise	After exercise
Glucose (mmol/l)	CONT	4.48 ± 0.40	4.15 ± 0.47	$3.97 \pm 0.41^{**}$
	HIT	5.12 ± 0.72	$4.49 \pm 0.50^{*}$	$4.35 \pm 0.46^{*}$
Lactate (mmol/l)	CONT	1.00 ± 0.37	$3.31 \pm 1.21^{***}$	$2.91 \pm 1.61^{**}$
	HIT	0.97 ± 0.18	$6.12 \pm 1.98^{***}$	$5.82 \pm 2.54^{***}$
Heart rate (bpm)	CONT	135.69 ± 15.00	$153.07 \pm 18.96^{*}$	$159.11 \pm 17.75^{*}$
	HIT	138.66 ± 13.59	$162.22 \pm 14.61^{**}$	$165.40 \pm 15.66^{**}$

Glucose and lactate levels in peripheral blood and heart rate before, during and after exercise (CONT: continuous exercise, HIT: high-intensity interval training). Glucose and Lactate measured periodically, heart rate measured continuously. Asterisks indicate statistically significant differences between before, during and after exercise (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$). Data are mean $\pm$ SD

to changes in heart rate, lactate and glucose levels (Table 2) as well as to leukocyte subsets, erythrocytes and platelets (Table 3, Additional file 2). Heart rate was increased significantly by both programs during and directly after exercise, while glucose levels decreased. The measured lactate levels were higher during exercise and began to decrease again directly after. Both CONT ($6.48 \times 10^3/\mu l \pm 1.38$ before exercise vs. 8.242 ± 1.50 1 h after exercise, $p < 0.01$) and HIT ($6.72 \times 10^3/\mu l \pm 1.19$ before exercise vs. $8.36 \times 10^3/\mu l \pm 2.32$ 1 h after exercise) increased the total count of leukocytes (white blood cells (WBCs)) in the standard CBC (Table 3, Panel A). All investigated immune cell subsets analysed in blood samples by using the haemocytometer, namely lymphocytes, granulocytes and monocytes (Table 3, Panel A, Additional file 2), indicated an increase of the absolute cell numbers 1 h after exercise, with recovery at least after 24 h. There was no significant difference between both training programs. Concerning the leukocytes, we found a higher increase of granulocytes compared to lymphocytes. This was confirmed by a slight decrease of the absolute lymphocyte count compared to an increase of the absolute leukocytes count (Table 3, Panel A). Similar results were obtained by single-cell flow cytometric analysis, investigating the frequency and absolute number of $CD45^+$ leukocyte and lymphocyte subsets (Table 3, Panel B; Additional file 2 and Additional file 3, shown as normalized data). Interestingly, the absolute number of $CD3^+$ T cells was increased continuously up to 24 h after exercise (Additional file 2E, shown as normalized data), while the CD4/CD8 T-cell ratio increased only slightly within 1 h after both training protocols and almost completely returned to pre-exercise levels within 24 h post training (Additional file 2F, shown as normalized data).

No significant differences between both training programs were identified with regards to the frequencies of the investigated cellular immune subsets ($CD45^+$ leukocytes, $CD45^+$ lymphocytes, $CD14^+$ monocytes, $CD19^+$ B cells, $CD3^+CD56^+$ NKT cells and $CD3^-CD56^+$ $NK^{dim/bright}$ cells (Additional file 3, shown as normalized data). The frequency of $CD14^+$ monocytes, was increased directly after both exercise programs, while the frequency of $CD19^+$ B cells increased within 1 h after both exercise programs and recovered within 24 h (Additional file 3, shown as normalized data). NK cell subsets displayed an inversely proportional trend (Additional file 3). The frequency of $CD3^-/CD56^{dim}$ NK cells increased within 1 h after both types of exercise, while the frequency of $CD3^-/CD56^{bright}$ NK cells decreased. Regarding donor fitness level, highly fit individuals had slightly higher frequencies of lymphocytes, in the resting state (Additional file 4A), while absolute numbers of white blood cells (WBC), lymphocytes, granulocytes and monocytes did not differ between donors with high and moderate fitness levels (Additional file 4B).

Exercise increased HSP70 levels while downregulating proliferation markers and not affecting replicative senescence

Quantitative real-time PCR showed that mRNA expression levels of HSP70 increased more than twofold within 24 h after HIT (2.9-fold), but only slightly after CONT exercise (up to 1.8-fold); the latter increases were detected directly and 1 h post exercise (Additional file 5A). The mRNA expression levels of the proliferation marker Ki67 were lower than baseline at all time points after both types of exercise. The mRNA levels of IFN-γ remained unchanged after CONT and increased slightly within 24 h after HIT. Granzyme B mRNA levels showed the same trend for both exercise programs: they increased slightly directly after exercise and then dropped to levels lower than starting point within 1 h and 24 h after exercise. The latter trend was more noticeable for CONT exercise. Telomeres were studied by quantification of telomere length, telomere ratio and

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Table 3 Total and differential leukocyte counts, total red blood cell and platelet counts

Parameter	Program	Before exercise	After exercise	1 h after exercise	24 h after exercise
Panel A					
Leukocytes ($\times 10^3$/µl]	CONT	6.48 ± 1.38	7.59 ± 1.52	8.24 ± 1.50**	7.02 ± 1.74
	HIT	6.72 ± 1.19	7.51 ± 2.08	8.36 ± 2.32	6.87 ± 1.15
Lymphocytes (%)	CONT	33.13 ± 8.10	31.97 ± 8.35	22.30 ± 6.58**	28.86 ± 8.86
	HIT	33.18 ± 8.40	30.14 ± 5.70	25.48 ± 6.84**	31.37 ± 7.98
Monocytes (%)	CONT	7.68 ± 8.1	7.59 ± 1.13	6.25 ± 1.42*	6.97 ± 1.64
	HIT	7.64 ± 1.74	7.57 ± 1.42	6.95 ± 1.03	7.58 ± 1.04
Granulocytes (%)	CONT	59.18 ± 8.17	60.28 ± 8.55	71.45 ± 7.15**	64.18 ± 9.43
	HIT	59.18 ± 8.98	61.84 ± 5.32	67.55 ± 7.42*	61.03 ± 8.62
Lymphocytes ($\times 10^3$/µl)	CONT	2.06 ± 0.39	2.38 ± 0.50	1.80 ± 0.47	1.81 ± 0.66
	HIT	2.18 ± 0.54	2.21 ± 0.61	2.07 ± 0.52	2.09 ± 0.51
Monocytes ($\times 10^3$/µl)	CONT	0.53 ± 0.14	0.58 ± 0.10	0.52 ± 0.13	0.48 ± 0.11
	HIT	0.52 ± 0.17	0.58 ± 0.20	0.59 ± 0.17	0.53 ± 0.11
Granulocytes ($\times 10^3$/µl)	CONT	3.88 ± 1.19	4.64 ± 1.36	5.93 ± 1.46**	4.63 ± 1.73
	HIT	3.99 ± 1.02	4.73 ± 1.43	5.75 ± 1.92*	4.17 ± 0.98
Red blood cell count ($\times 10^6$/µl)	CONT	5.25 ± 0.94	5.30 ± 0.64	5.26 ± 0.99	5.299 ± 1.09
	HIT	5.37 ± 0.64	5.44 ± 0.82	5.15 ± 0.91	5.028 ± 0.90
Hemoglobin [g/dl]	CONT	15.36 ± 2.85	15.44 ± 1.97	15.32 ± 2.95	15.50 ± 3.24
	HIT	15.69 ± 2.49	15.78 ± 2.30	14.96 ± 2.59	14.63 ± 2.55
Hematocrit [%]	CONT	46.84 ± 8.41	47.00 ± 5.54	46.48 ± 8.49	46.92 ± 9.38
	HIT	47.81 ± 6.43	47.99 ± 6.78	45.41 ± 7.69	44.43 ± 7.56
Platelet count ($\times 10^3$/µl)	CONT	185.80 ± 67.21	213.20 ± 75.70	187.30 ± 72.87	176.30 ± 72.45
	HIT	185.10 ± 48.55	203.40 ± 65.69	178.80 ± 53.26	186.80 ± 54.98
Plateletcrit (%)	CONT	0.17 ± 0.05	0.19 ± 0.06	0.16 ± 0.06	0.15 ± 0.06
	HIT	0.17 ± 0.05	0.18 ± 0.06	0.16 ± 0.05	0.16 ± 0.05
Parameter	**Program**	**Before exercise**	**After exercise**	**1 h after exercise**	**24 h after exercise**
Panel B					
CD45+ Leukocytes (/µl)	CONT				
	Mean	1.12E+04	1.26E+04	1.33E+04	1.20E+04
	SD	1.48E+04	1.81E+04	1.91E+04	2.26E+04
	HIT				
	Mean	9.37E+03	1.19E+04	1.31E+04	6.20E+03
	SD	8.50E+03	1.44E+04	1.58E+04	9.41E+02
CD45+ Lymphocytes (%)	CONT				
	Mean	32.19	32.00	23.63**	34.15
	SD	7.22	7.20	4.82	6.76
	HIT				
	Mean	31.50	29.22	23.84	31.22
	SD	8.08	5.78	6.13	7.14
CD45+ Lymphocytes (/µl)	CONT				
	Mean	2.17E+03	2.26E+03	1.88E+03	1.95E+03
	SD	5.90E+02	5.51E+02	4.53E+02	6.28E+02
	HIT				
	Mean	2.09E+03	2.15E+03	1.91E+03	1.93E+03
	SD	5.65E+02	5.83E+02	6.02E+02	5.29E+02

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Table 3 (continued)

Parameter	Program	Before exercise	After exercise	1 h after exercise	24 h after exercise
CD3$^+$ T cells (%)	CONT				
	Mean	74.97	75.57	78.45	77.74
	SD	5.20	6.51	5.49	5.62
	HIT				
	Mean	75.81	76.29	77.25	77.62
	SD	4.27	5.16	5.29	4.78
CD3$^+$ T cells (/µl)	CONT				
	Mean	1.62E+03	1.71E+03	1.48E+03	1.53E+03
	SD	4.34E+02	4.58E+02	3.87E+02	5.38E+02
	HIT				
	Mean	1.58E+03	1.64E+03	1.48E+03	1.50E+03
	SD	4.02E+02	4.53E+02	4.63E+02	4.19E+02

Cell counts in peripheral blood (Panel A) analysed by using a haemocytometer as well as (Panel B) total leukocyte counts, total and proportional lymphocyte and T-cell counts analysed by flow cytometry before, directly after, 1 h after and 24 h after 30 min of continuous (CONT) and high-intensity interval (HIT) cycling exercise (n = 12). Statistically significant difference from before exercise indicated by (*p < 0.05 and **p < 0.01). Data are mean ± SD

p16 expression (Additional file 5B). None of these three parameters changed significantly in terms of both exercise programs and sample time, even though the telomere ratio tended to be lower 24 h after HIT and CONT exercise.

HIT had the biggest impact on the number of functionally active VSTs

The exercise-induced change in the number of functionally active VSTs against the investigated peptide pools derived from CMV, EBV and AdV was measured by IFN-γ EliSpot assay at different time points before and after HIT and CONT (Fig. 2). The positive control showed a positive result for all analysed samples specified as TNTC (too numerous to count, TNTC ≥ 1000 spw). Overall, donors exhibited a higher increase 24 h after HIT than 24 h after CONT exercise, which induced almost no or minimal change. The change in the number of functionally active VSTs, expressed as the number of spots per 1000 CD3$^+$ T cells from before to 24 h after exercise, was 1.2-fold CMV pp65, 2.6-fold CMV IE-1 (Fig. 2a), 1.3-fold EBV EBNA1, 1.2-fold EBV Consensus (Fig. 2b), 1.6-fold AdV5 Hexon, 2.2-fold AdV5 Penton (Fig. 2c) for HIT compared to 1.0-fold CMV pp65, 2.7-fold CMV IE-1, 0.7-fold EBV EBNA1, 0.7-fold EBV Consensus 0.7-fold, 0.8-fold AdV5 Hexon and AdV5 Penton for CONT. Even though this trend was strong, it only reached statistical significance with AdV5 Hexon directly after HIT exercise, and a 1.6-fold increase occurred within 24 h (p < 0.05, Fig. 2c).

Comparing the effect of HIT and CONT with the two-sample t approach adjusting for period, there was no significant difference between the programs except for AdV5 Penton and EBV Consensus 24 h after exercise (p = 0.013 and p = 0.006), where HIT had the bigger effect (Additional file 6).

Concerning the effects of the individual donor fitness level on VST activation (Fig. 3), HIT most effectively boosted VSTs against AdV- and EBV-derived antigens in moderately fit donors (AdV: up to 2.5-fold increased; EBV: up to 2.8-fold increased) and was less efficient in augmenting the immunodominant CMV-derived antigen pp65 (2.5-fold increased) than the less immunodominant CMV IE1 (4.5-fold increased).

Proof-of concept: enrichment of antiviral T cells using small scale Cytokine Secretion Assay

Encouraged by the positive effect of HIT on VST activation identified by the IFN-γ EliSpot assay, we also examined its effect on IFN-γ-secreting T cells using the IFN-γ CSA, which revealed a similar outcome (Fig. 4). All antigens except the highly immunodominant antigen CMV pp65 [46] evoked fold increases in CD3$^+$ T cells after short stimulation with the largest increased detected 24 h after exercise. The fold increases at 24 h after exercise compared to baseline were as follows: CD3$^+$/IFN-γ$^+$: 0.73-fold CMV pp65, 1.8-fold CMV IE-1, 2.5-fold EBV EBNA1, 1.4-fold EBV Consensus, 2.3-fold AdV5 Hexon and 5.3-fold AdV5 Penton.
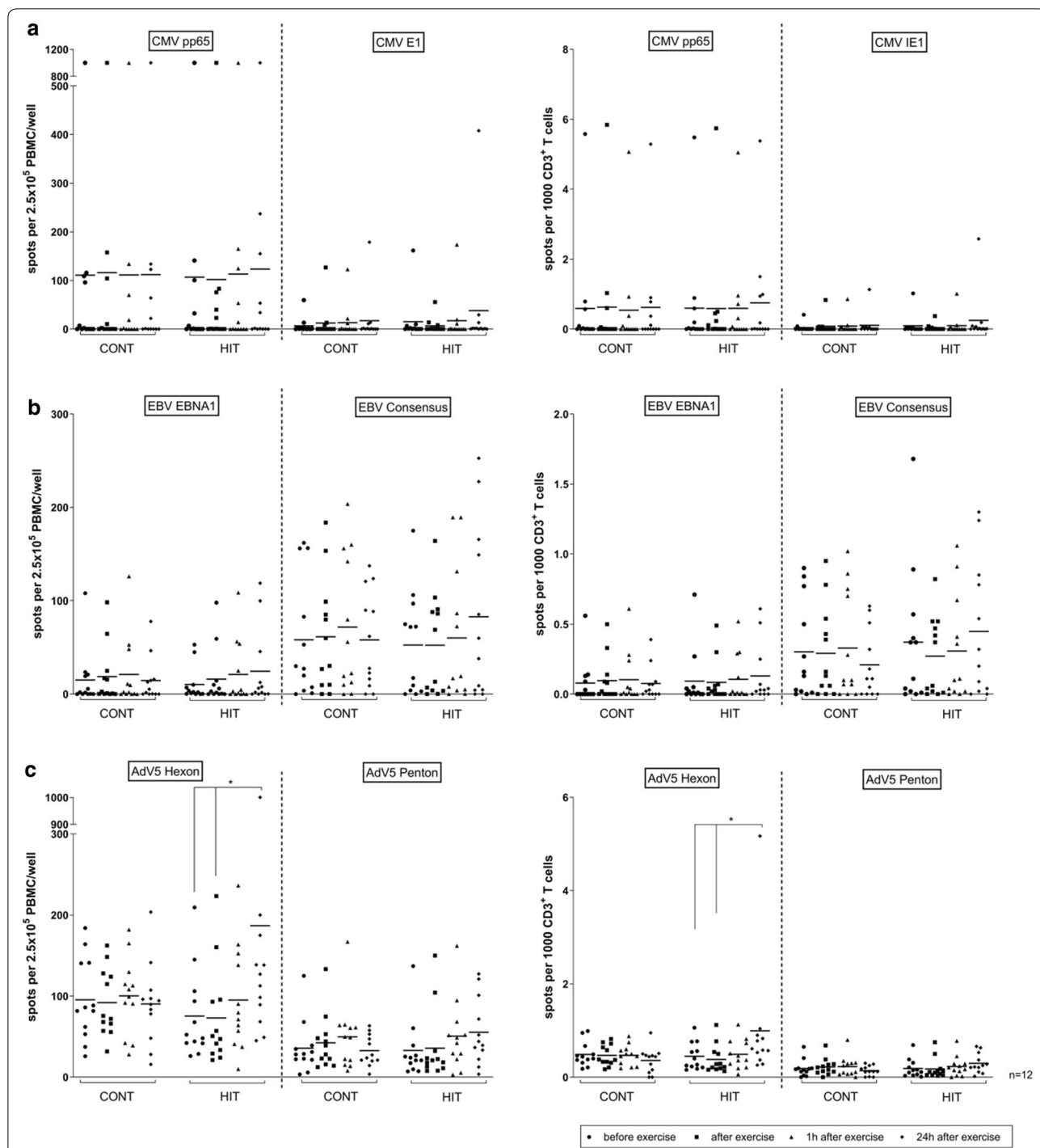
Fig. 2 Impact of a single continuous and interval exercise on antigen-specific T-cell responses. Peripheral blood samples of healthy donors (n = 12) were analysed at different time points before and after a single 30 min continuous (CONT) or interval (HIT) exercise (before, directly after, 1 h after and 24 h after exercise). Isolated PBMCs were stimulated overnight with **a** CMV-, **b** EBV- and **c** AdV-specific peptide pools (CMV pp65, CMV IE1, EBV EBNA1, EBV Consensus, AdV5 Hexon and AdV5 Penton) and frequencies of functional-active virus-specific T cells were determined by IFN-γ EliSpot assay. Negative and positive controls were carried out by using either medium without stimuli or 1 μg/ml staphylococcal enterotoxins B (SEB). Results are indicated as the number of spots per 2.5×10^5 cells/well (spw) and spots per 1000 CD3$^+$ T cells, respectively after substracting the number of spw of the respective negative control. Positive controls showed a positive result for all analysed samples detected as TNTC (too numerous to count, TNTC ≥ 1000 spw). Results are displayed as mean ± SD. Asterisks indicate statistically significant differences between levels of induced cytokine responses (*p < 0.05)

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Overall, AdV5 Penton exhibited the greatest increase (CD3$^+$/IFN-γ$^+$: 5.3-fold increase 24 h after exercise, CD8$^+$/IFN-γ$^+$: 12.4-fold increase 24 h after exercise, CD4$^+$/IFN-γ$^+$: 3.4-fold increase 24 h after exercise).

A single bout of exercise directly affected CD8$^+$ T-cell cholesterol and activation levels

A single bout of CONT or HIT resulted in a steady increase in cholesterol in isolated CD8$^+$ T cells (Fig. 5). The overall change from before to 24 h after exercise was 1.46-fold (Fig. 5). This change correlated to expression levels of the activation marker CD69 on isolated CD8$^+$ T cells; the total change was 1.66-fold before to 24 h after exercise (Fig. 5b). At 24 h post-exercise, the increase in cholesterol was higher after CONT exercise (1.67-fold increase, CD69: 1.66-fold change) than after HIT (1.29-fold change, CD69: 1.69-fold change). Donor-related comparison confirmed these results: individual donors showed higher increases in cholesterol 24 h after CONT (1.92-fold change) than 24 h after HIT (1.09-fold change), which only led to a slight increase in cholesterol (Additional file 7). Regarding the relationship between donor fitness level and cholesterol (Fig. 5c), the highest cholesterol levels after a single bout of HIT exercise were detected in donors with a high fitness level (2.40-fold change 1 h after exercise), whereas those observed after a single bout of CONT exercise occurred in donors with a moderate fitness level (1.96-fold change 1 h after exercise).

Discussion

Research has shown that physical exercise augments the number of circulating VSTs in peripheral blood [41]. The current study demonstrates for the first time that (1) HIT is the preferable type of exercise for this purpose, (2) the optimal time for efficient augmentation is 24 h prior to leukapheresis, and (3) HIT most effectively boosts the proliferation of VSTs with low resting frequencies in moderately fit donors. These results may have important implications for VST production with regard to coaching donors prior to leukapheresis.

Jamurtas et al. [52] recently observed that WBC and lymphocyte counts increase immediately after both continuous exercise and high-intensity interval training, while monocyte counts remain unchanged. The fact that the increase after HIT was significantly greater suggests that the VST yield may also be augmented post interval training. In agreement with our results, the observed leuko- and lymphocytosis returned to baseline within 24 h after training. Walsh et al. reported that the same biphasic pattern of mobilization can be observed for T cells and is mainly due to the direct and indirect effects of epinephrine [40]. It has been shown that a single bout of various types of continuous exercise mobilizes VSTs specific for latent and non-latent herpesviruses (i.e. CMV, EBV and AdV) to the peripheral bloodstream and has a beneficial effect on their in vitro expansion [25, 41].

Accordingly, we also observed an increase in leukocyte and lymphocyte counts directly after both exercise protocols, while the number and function of CD3$^+$ T cells was highest 24 h after exercise. This response could be due to the sympathetically mediated elevation of stress levels directly after exercise resulting in higher glucocorticoid production and, therefore, temporary inhibition of cytokine production until recovery [53].

Strikingly, we observed the highest T-cell response after 30 min of HIT against AdV5 Hexon and AdV5 Penton in both IFN-γ EliSpot Assay and IFN-γ CSA, while CMV pp65 did not respond as much to exercise. HIT appears to augment IFN-γ secretion, especially in the case of circulating memory VSTs with low precursor frequencies, like AdV-specific T cells [46]. Conversely, T cells against the highly immunodominant antigen CMV pp65 [54], which have higher frequencies in the circulation of healthy donors, did not respond as much to exercise. This finding suggests that HIT is most beneficial for boosting antigen-specific T cells with low precursor frequencies.

Interestingly, these beneficial effects on mobilization and function were more distinct in moderately fit individuals. It is known that regular prolonged exercise promotes immunosenescence of T cells [55]. This could be

(See figure on next page.)
Fig. 3 Changes in frequencies of VSTs between moderately and highly fit donors. Donors were additionally classified into a moderately and a highly fit group according to the International Physical Activity Questionnaire (IPAQ). Frequencies of functional-active virus-specific T cells were determined by IFN-γ EliSpot assay. Negative and positive controls were carried out by using either medium or 1 µg/ml staphylococcal enterotoxins B (SEB). Results **a** in response to CMV, **b** EBV and **c** AdV from before and 24 h after exercise are shown as fold changes of spots per well per 1000 CD3$^+$ T cells after subtracting those of the respective negative control. Positive control showed a positive result for all analysed samples detected as TNTC (too numerous to count, TNTC ≥ 1000 spw). Results are displayed as mean ± SD

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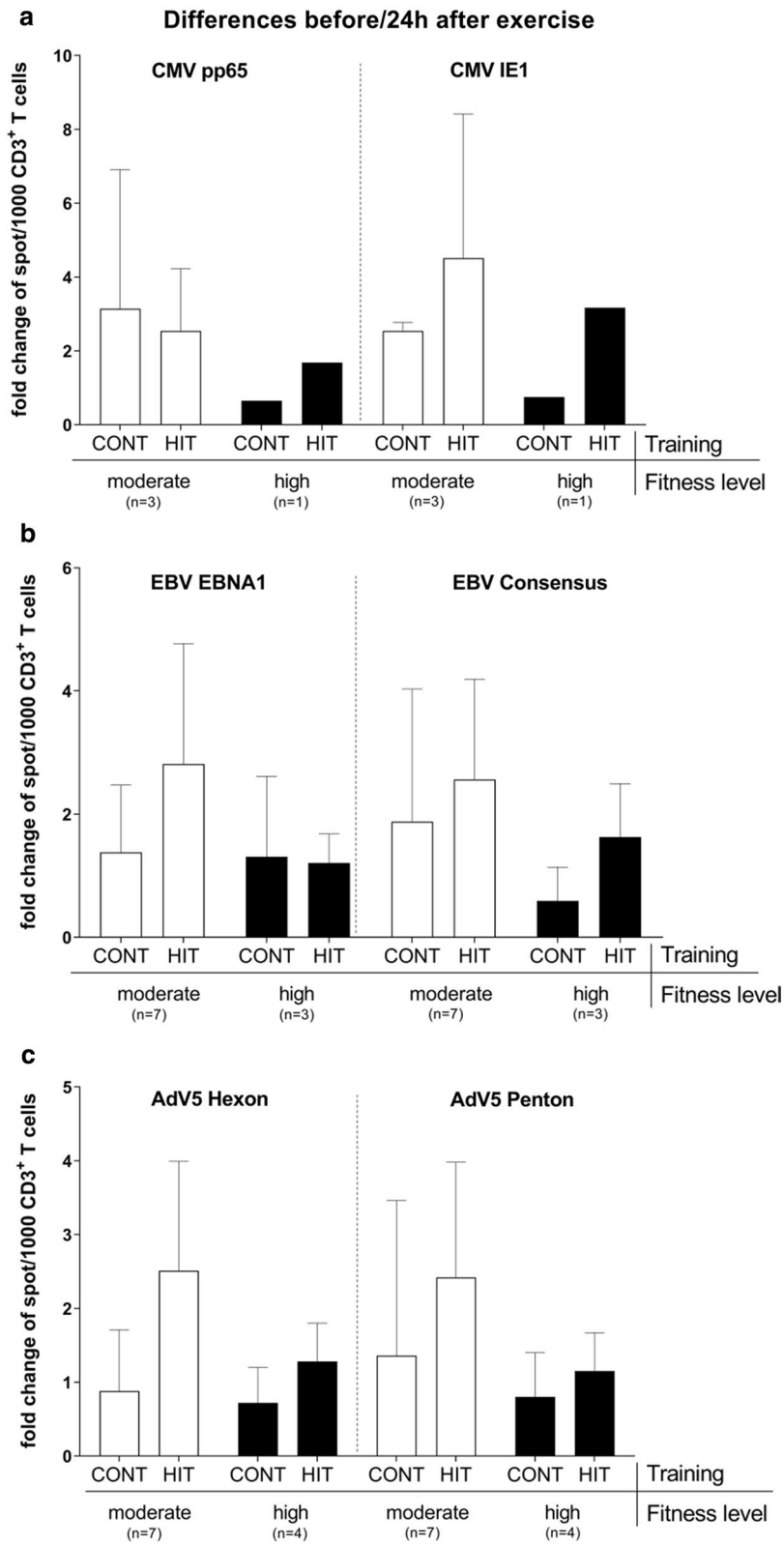
a reason why the highly fit individuals did not respond as much to a single session of exercise. Another reason could be that highly fit individuals are more used to exercise stimuli and therefore have a lower VST activation response to exercise.

Among other co-stimulatory factors, a key component of this activation response is the clustering of T-cell receptors (TCR) at the centre of the T-cell/antigen-presenting-cell interface [56]. Recent studies have demonstrated that enhanced cholesterol levels of the whole cell and the plasma membrane are linked to increased functionality of activated $CD8^+$ T cells resulting in enhanced TCR clustering and signalling as well as more efficient formation of the immunological synapse [57–60]. For this reason, we investigated whether a single bout of HIT or CONT results in higher cholesterol levels, which could lead to increased activation and cytotoxic function of $CD8^+$ T cells by strengthening TCR signaling. Indeed, cholesterol levels in $CD8^+$ T cells increased in response to exercise. Even though the difference between the two exercise protocols was not statistically significant, cholesterol levels and CD69 expression in $CD8^+$ T cells increased significantly after continuous exercise, predominantly in moderately fit donors. As early marker for VSTs activation [61], the observed increases in CD69 and cholesterol correlated well with higher IFN-γ secretion. In a comprehensive future study, we will investigate the impact and modulatory role of cholesterol on VST activation and function.

Another interesting trend could be seen with HSP70. Different types of stress, one of which is exercise, induces HSP70 expression in order to adapt organisms appropriately [62, 63]. In a rat model, it was shown that at a high-intensity workload, HSP70 increases while the proliferation response decreases [62]. This corresponds to our findings concerning HSP70 and the proliferation marker Ki67.

However, it is not surprising that a single bout of exercise did not alter measures of replicative senescence such as telomere length. Habitual exercise slows the age-related decrease in telomere length. However, this seems to be one of the long-term effects of regular exercise [64] and is not observed after a single exercise bout.

Although they are now used as second-line treatment in patients who do not respond to first-line treatments such as cidofovir for AdV infections, researchers are evaluating the usefulness of VSTs as an adjuvant to standard agents in the initial treatment [65].

In the present study, the peak VST frequencies differed from the peak levels of general lymphocyte counts: HIT did not evoke the highest VST activity when the $CD4^+$ and $CD8^+$ frequencies peaked. In fact, a higher antigen response was observed 24 h after HIT even though frequencies of the above-mentioned T-cell subsets were higher at other time points. Spielmann et al. found that although continuous exercise augments the ex vivo manufacture of VSTs, this effect does not appear to be due to numerical changes in the number of VSTs stimulated; hence, they deemed it unlikely that the beneficial effect of exercise achieved in their ex vivo expansion experiments could be replicated by simply drawing larger volumes of blood [39].

Thus, a single bout of HIT exercise 24 h before donation can serve as a simple and economical adjuvant to significantly improve adoptive T-cell therapy with respect to manufacturing functional-active antigen-specific T-cell products in sufficient cell numbers. This is especially effective in the case of memory VSTs with low precursor frequencies.

Conclusions

A single bout of exercise, preferably HIT 24 h prior to blood donation, can significantly improve T-cell enrichment in VST production for adoptive T-cell immunotherapy. Exercise has benefits, especially for viral antigens for which most healthy individuals possess less frequent T cells, particularly in donors who are moderately fit. As single bouts of exercise in general seem to be beneficial, they can be recommended to all donors.

(See figure on next page.)
Fig. 4 Effects. of a single continuous and interval exercise on the activation capacity of functional IFN-γ$^+$ antigen-specific T cells. Peripheral blood samples of healthy donors (n = 6) were analysed before, 1 h after and 24 h after a single 30 min interval (HIT) using IFN-γ Cytokine Secretion Assay (CSA). Isolated PBMCs were stimulated overnight with **a** CMV-, **b** EBV- and **c** AdV-specific peptide pools (CMV pp65, CMV IE1, EBV EBNA1, EBV Consensus, AdV5 Hexon and AdV5 Penton). Negative control was carried out by using unstimulated PBMCs. The relevant cell fractions before and after enrichment were used for a detailed flow cytometric analysis of IFN-γ$^+$ T-cell subsets. Results are shown as fold changes of the frequency of $CD3^+$/IFN-γ$^+$, $CD8^+$/IFN-γ$^+$ and $CD4^+$/IFN-γ$^+$ cells after subtracting those of the respective negative control. CMV and AdV results are displayed as mean ± SD
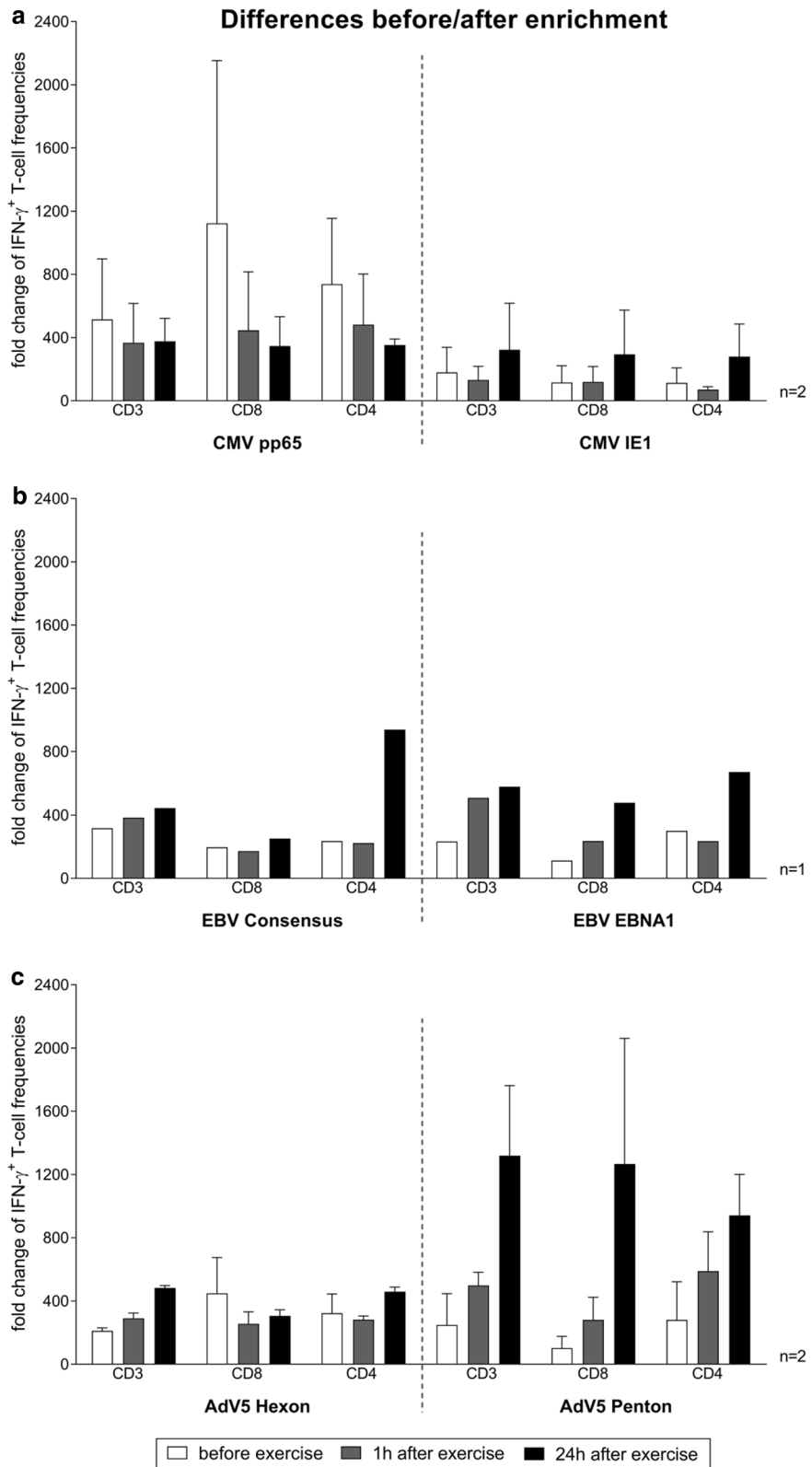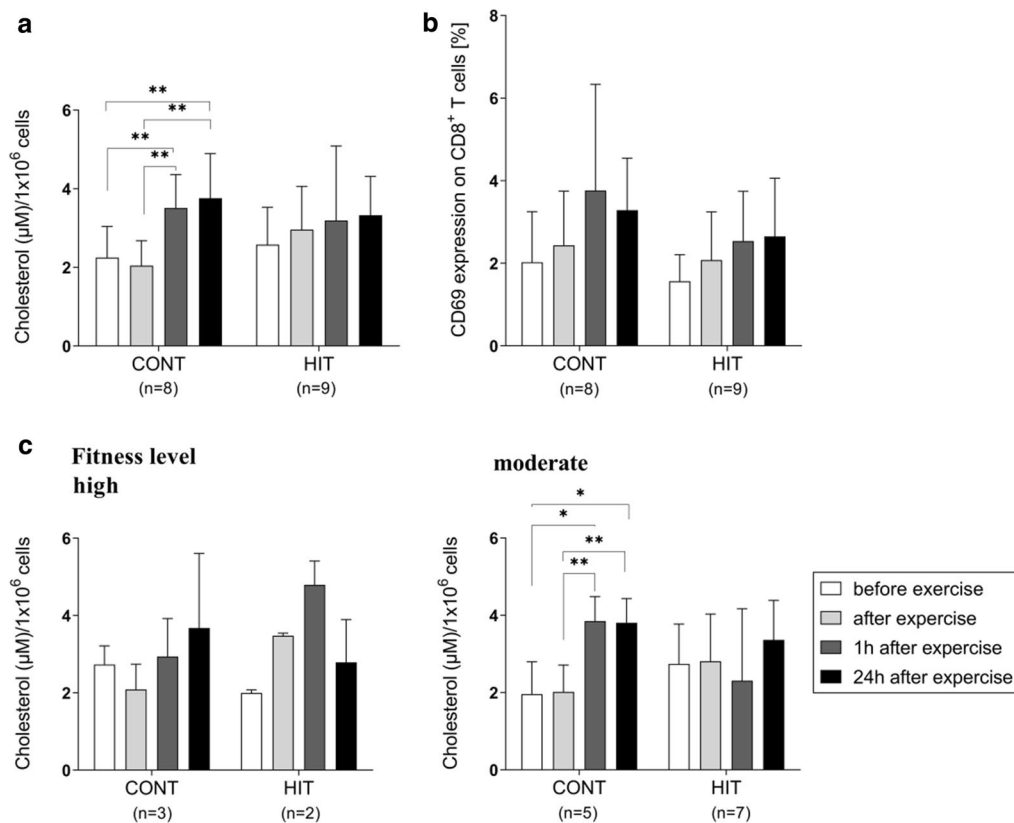
Fig. 5 Effects of a single continuous and interval exercise on CD8+ T-cell cholesterol and activation levels. Peripheral blood samples of healthy donors (n = 12) were analysed at different time points before and after exercise (before, directly after, 1 h after and 24 h after exercise), in which each donor at least completed one training program (CONT, 8/12 donors and HIT, 9/12 donors) to quantify the level of cholesterol and to analyse the CD69 expression on CD8+ T cells. Data are shown in total as mean ± SD. Statistically significant difference is indicated by (*p < 0.05) and (**p < 0.01). Cholesterol and cell activation related parameters were obtained as **a** Cholesterol (μM)/1 × 10^6 cells (Amplex Red Cholesterol Assay Kit) and **b** CD69 expression on CD8+ T cells (flow cytometry). **c** Additionally, the cholesterol level was analysed regarding the donors' fitness level. 4/12 donors were classified as donors with a high fitness levels (CONT n = 3/4 and HIT n = 2/4) and 8/12 as donors with a moderate fitness level (CONT n = 5/8 and HIT n = 7/8)

Supplementary information

Additional file 1. Representative flow cytometric plots. Representative flow cytometry plots illustrating the gating strategy used to analyse (A) the cellular immune status in whole blood sample on a single-cell platform using TruCount tubes, (B) the enrichment efficiency of IFN-γ+ VSTs selected from PBMCs using the IFN-γ cytokine secretion assay, and (C) the activation level of isolated CD8+ T cells using CD69 as specific marker before and after exercise.

Additional file 2. Changes on the cellular immune status after continuous and interval exercise. Peripheral blood samples of healthy donors (n = 12) were analysed at different time points before and directly after a single 30 min continuous (CONT) or interval (HIT) exercise (before, directly after, 1 h after and 24 h after exercise) using a haemocytometer (A–D) or flow cytometry (E–F). (A) White blood cell (WBC), (B) lymphocyte, (C) granulocyte and (D) monocyte counts were detected as absolute counts (×10^3/μl). Data are shown as normalized cell counts (A–D), normalized frequencies of (E) CD3+ T cells and the (F) normalized CD4/CD8 ratio. Data are mean ± SD.

Additional file 3. Changes on standard blood counts and the cellular immune status after continuous and interval exercise. Peripheral blood samples of healthy donors (n = 12) were analysed by flow cytometry at different time points before and after a single 30 min continuous (CONT) or interval (HIT) exercise (before, directly after, 1 h after and 24 h after exercise). Determined frequencies of CD45+ leukocytes, CD14+ monocytes, CD19+ B cells, CD3+/CD56+ NKT cells, and CD3−/CD56+ NK cells with their bright and dim subsets are displayed as normalized frequencies. Data are mean ± SD.

Additional file 4. Changes in standard blood counts after continuous and interval exercise differentiating between moderately and highly fit donors. Peripheral blood samples of healthy donors (n = 12) were analysed using a haemocytometer at different time points before and after a single 30 min continuous (CONT) or interval (HIT) exercise (before, directly after, 1 h after and 24 h after exercise). Donors were grouped according to their fitness level (moderate, n = 8, and high, n = 4, tested with the International Physical Activity Questionnaire, IPAQ). (A) Lymphocyte, granulocyte, and monocyte frequency is shown as percentage of the total leukocyte count. (B) White blood cell (WBC), lymphocyte, granulocyte, and monocyte counts are shown as absolute counts (×10^3/μl blood). Data are shown as mean ± SD.

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Additional file 5. Effects of a single continuous and interval exercise on stress, proliferation markers, T-cell cytotoxicity and parameters of cellular age. Peripheral blood samples of healthy donors (n = 12) were analysed at different time points before and after a single 30 min continuous (CONT) or interval (HIT) exercise (before, directly after, 1 h after and 24 h after exercise) using real-time PCR for quantification of (A) HSP70- and Ki67-, IFN-γ-, and granzyme B-mRNA levels. Constitutively expressed GAPDH gene was used as the reference standard for normalization of mRNA levels. RQ values were calculated by the delta–delta CT method. (B) Blood samples were further analysed regarding p16 levels, telomere ratio and telomere base pairs. Results are displayed as RQ, with the value from before exercise being the base line, and shown as mean ± SD.

Additional file 6. Impact of a single continuous and interval exercise on antigen-specific T-cell responses. Peripheral blood samples of healthy donors (n = 12) were analysed separately with the two-sample t approach adjusting for period at different time points before and after a single 30 min continuous (CONT) or interval (HIT) exercise (before, directly after, 1 h after and 24 h after exercise). Isolated PBMCs were stimulated overnight with CMV-, EBV- and AdV-specific peptide pools (CMV pp65, CMV IE1, EBV EBNA1, and EBV Consensus, AdV5 Hexon, and AdV5 Penton) and frequencies of functional-active virus-specific T cells were determined by IFN-γ EliSpot assay as spots per 1000 CD3$^+$ T cells

Additional file 7. Effects of a single continuous and interval exercise on CD8$^+$ T-cell cholesterol and activation levels in a donor-related setting. Peripheral blood samples of healthy donors (n = 5) were analysed at different time points before and after a single 30 min continuous (CONT) or interval (HIT) exercise (before, directly after, 1 h after and 24 h after exercise) to (A) quantify the level of cholesterol (μM)/1 × 10^6 cells (Amplex Red Cholesterol Assay Kit) and (B) to obtain the expression of CD69 on CD8$^+$ T cells (flow cytometry). Data are shown in a donor-related setting as mean ± SD. Statistically significant difference is indicated by (*p < 0.05) and (**p < 0.01).

Abbreviations
AdV: Adenovirus; APC/Cy7: Anti-CD45-allophycocyanin plus cyanin-7; CBC: Complete blood counts; CM: Culture medium; CMV: Cytomegalovirus; CONT: Continuous exercise; CSA: Cytokine Secretion Assay; DLI: Donor lymphocyte infusions; EBV: Epstein–Barr-virus; EliSpot assay: Enzyme-linked immunospot assay; FITC: Anti-CD3-fluorescein isothiocyanate; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; GvHD: Graft-versus-host disease; GXT: Graded exercise test; HHV6: Human herpesvirus 6; HIHVT: High Intensity High Volume Training; HIT: High Intensity Interval Training; HR: Heart rate; HSCT: Hematopoietic stem cell transplantation; HSP70: Heat shock protein 70; IFN-γ: Interferon-γ; IPAQ: International Physical Activity Questionnaire; mAbs: Monoclonal antibodies; MHH: Hannover Medical School; NK cells: Natural killer cells; PBMCs: Peripheral blood mononuclear cells; PE: Phycoerthrin; PerCP: Peridinin chlorophyll protein; P$_{max}$: Maximum power output; pMHC: Peptide major histocompatibility complex; PTLD: Post-transplant lymphoproliferative disorder; qPCR: Quantitative real-time PCR; SEB: Staphylococcal enterotoxin B; spw: Spots per well; SOT: Solid organ transplantation; TCR: T-cell receptors; VST: Virus-specific T cells; WBC: White blood cells; 7AAD: 7-Amino-actinomycin D.

Authors' contributions
NCH recruited all participants, assisted with exercise testing, designed and performed T-cell stimulation experiments and functional assays, contributed to data analysis, and co-wrote the manuscript. STZ helped to design the study, performed T-cell stimulation assays and immunoassays, contributed to the data analysis, and co-wrote the manuscript. TCW, JE and AK drafted and executed exercise testing and provided sports medicine expertise. TF provided helpful input to discussions concerning statistical analysis. AM designed and performed the replicative senescence experiments. HGH contributed with helpful input to discussions. BMK contributed helpful input to discussions and assisted in exercise testing. RB contributed helpful input to discussions. BEV conceived the study, contributed to its design and coordination, helped to perform the experiments and co-wrote the manuscript. All authors read and approved the final manuscript.

Availability of data and materials
All data generated or analysed during this study are included in this published article and its additional files. The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The study was approved by the Institutional Review Board of Hannover Medical School (MHH, approval number 3366-2016). After giving their written informed consent, each participant underwent a medical check-up to exclude active infections and an electrocardiogram in rest was evaluated by an experienced cardiologist to make sure that participants could join the exhaustion test.

Consent for publication
We declare that all authors concur with the submission of this manuscript.

Competing interests
None of the authors of the given manuscript has any conflicts of financial interest to disclose regarding the aforementioned study. The authors declare that they have no competing interests.

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