Aus der Klinik für Neurologie mit experimenteller Neurologie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Efficacy and harms of a four week aerobic gait training
compared to relaxation therapy in patients with subacute
stroke

Wirksamkeit und Nebenwirkungen eines vierwöchigen
aeroben Gangtrainings im Vergleich zu einer
Entspannungstherapie bei Patienten nach subakutem
Schlaganfall

zur Erlangung des akademischen Grades
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von

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To my wife
and my children

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List of abbreviations

ANCOVA: Analysis of covariance

CI: Confidence interval

CT: Computer tomography

Df: Degrees of freedom

DSMB: Data safety monitoring board

EMA: European Medicines Agency

FAC: Functional Ambulatory Category

FAQ: Frequently asked questions

FDA: Food and Drug Administration

FLAIR: Fluid attenuated inversion recovery

ICV: Intracranial volume

IRR: Incidence rate ratios

IQR: Interquartile range

LAA: Large artery artherosclerosis

MAR: Missing at random

MPRAGE: Magnetization-prepared rapid gradientecho

MRI: Magnetic resonance imaging

NIHSS: National Institute of Stroke Scale

SAE: Serious adverse events

SD: Standard deviation

TE: Echo time

TR: Repetition time

TI: Inversion time

VO^2: Oxygen consumption

1. Abstract

<u>English</u>

Introduction: Despite ongoing research no pharmaceutical means has proven efficacious to improve functional recovery in stroke patients. One promising therapeutic intervention is aerobic gait training as it is hypothesized to improve systemic oxygenation and cerebral recovery processes while being functional. For the early subacute phase after stroke, first exploratory studies could show an increase in mobility and improved functionality through aerobic training but feasibility in this high-risk population remains unclear. In this study we aimed to address the effect of an aerobic gait training compared to an active control intervention in the subacute phase after stroke and assess its feasibility, including safety, training adherence and treatment fidelity.

Methods: The multicenter, randomized controlled, endpoint-blinded trial 'Physical Fitness Training in Patients with Subacute Stroke – Phys-Stroke' recruited 200 patients in the subacute phase (5 – 45 days) after moderate to severe stroke. Participants were randomized (1:1) to receive either a four week aerobic, bodyweight supported gait training (n = 105) with five sessions per week á 25 minutes or a progressive muscle relaxation therapy (n = 95) of equal amount. Co-primary efficacy endpoints were changes in maximal walking speed and in Barthel index score at three months post stroke. Secondary endpoints included six minute walking distance, gait energy cost and change in lesion volume. Safety measures were assessed until six months post stroke and included the serious adverse events (SAE) recurrent cerebro- or cardiovascular event, referral to an acute hospital or death. Adjusted analysis of co-variance were used to analyze both primary endpoints independently in the full analysis set. Poisson regression analysis were performed to assess incidence rate ratios (IRR) of SAE between both intervention groups.

Results: Compared to relaxation therapy, aerobic training did not improve maximal walking speed (adjusted treatment effect 0.1 m/s (95% confidence interval 0.0 to 0.2 m/s), p=0.23) or Barthel index score (0, 95% CI −5 to 5, p=0.99) significantly at three months post stroke. Exploratory findings revealed an interaction effect of female sex with training (0.3 m/s, 95% CI 0.1 to 0.5) and a treatment effect of change in lesion volume (-21.8 ml, 95% CI -40.4 to -3.2). Incidence rates (per 100 patient months) were

higher in the training group compared to relaxation for all SAE combined (6.31 vs 3.22; IRR 1.70, 95% CI 0.96 to 3.12).

Discussion: Aerobic training delivered in the subacute phase after moderate to severe stroke was not found to be superior on maximal walking speed or activities of daily living compared to relaxation therapy. Additionally, a higher rate of SAE in the training group questions feasibility of aerobic exercise in this stroke population. Results are in contrast to previous smaller studies and should be considered in future guidelines.

<u>German</u>

Einleitung: Bisher hat sich kein pharmazeutisches Mittel zur Verbesserung der funktionellen Erholung bei Schlaganfallpatient:innen als wirksam erwiesen. Eine vielversprechende therapeutische Intervention ist das aerobe Gangtraining. Für die frühe subakute Phase nach Schlaganfall konnten erste explorative Studien eine Steigerung der Mobilität und eine verbesserte Funktionalität durch aerobes Training zeigen, aber die sichere Durchführung in dieser Hochrisikopopulation bleibt unklar. Ziel dieser Studie war es, den Effekt eines aeroben Gangtrainings im Vergleich zu einer aktiven Kontrollintervention in der subakuten Phase nach Schlaganfall zu untersuchen und dessen Durchführbarkeit, einschließlich der Sicherheit, Trainings- und Therapietreue zu bewerten.

Methoden: Die multizentrische, randomisierte, kontrollierte, Studie 'Physical Fitness Training in Patients with Subacute Stroke - Phys-Stroke' rekrutierte 200 Patient:innen in der subakuten Phase (5 - 45 Tage) nach moderatem bis schwerem Schlaganfall. Die Teilnehmer:innen wurden randomisiert (1:1), um entweder ein vierwöchiges aerobes, körpergewichtsunterstütztes Gangtraining (n = 105) mit fünf Sitzungen pro Woche á 25 Minuten oder eine gleichwertige progressive Muskelentspannungstherapie (n = 95) zu erhalten. Ko-primäre Endpunkte waren Veränderungen in der maximalen Ganggeschwindigkeit und im Barthel-Index drei Monate nach dem Schlaganfall. Zu den sekundären Endpunkten gehörten die sechs Minuten Gehstrecke, der Energieaufwand beim Gehen und die Veränderung des Läsionsvolumens. Sicherheitsendpunkte wurden bis sechs Monate nach dem Schlaganfall erhoben und umfassten die schwerwiegenden unerwünschten Ereignisse (SUE) rezidivierendes zerebro- oder kardiovaskuläres Ereignis, Einweisung in ein Akutkrankenhaus oder Tod.

Ergebnisse: Im Vergleich zur Entspannung verbesserte das aerobe Training bis drei Monate nach dem Schlaganfall weder die maximale Ganggeschwindigkeit (adjustierter Behandlungseffekt 0,1 m/s (95% Konfidenzintervall 0,0 bis 0,2 m/s), p=0,23) noch den Barthel-Index (0 (-5 bis 5), p=0,99) signifikant. Explorative Ergebnisse zeigten einen Interaktionseffekt des weiblichen Geschlechts mit dem Training (0,3 m/s, 95% CI 0,1 bis 0,5) und einen Behandlungseffekt auf die Veränderung des Läsionsvolumens (-21,8 ml, 95% CI -40,4 bis -3,2). Die Inzidenzraten (pro 100 Patientenmonate) waren in der Trainingsgruppe im Vergleich zur Entspannungsgruppe für alle SUE zusammen höher (6,31 *vs.* 3,22; IRR 1,70, 95% CI 0,96 bis 3,12).

Diskussion: Aerobes Training in der subakuten Phase nach mittelschwerem bis schwerem Schlaganfall erwies sich im Vergleich zur Entspannungstherapie nicht als überlegen in Bezug auf die maximale Ganggeschwindigkeit oder Aktivitäten des täglichen Lebens. Zusätzlich stellt eine höhere Rate an SUE in der Trainingsgruppe die Durchführbarkeit von aerobem Training in dieser Population in Frage.

2. Introduction

Cerebral stroke is defined as an "episode of acute neurological dysfunction" which is caused by ischemia or hemorrhage unrelated to a traumatic event, or any evidence of infarction found by imaging or pathological findings as endorsed by the American Heart Association and the American Stroke Association.[1] The majority of risk factors for ischemic stroke are modifiable such as hypertension, hyperlipidemia, current smoking, waist-to-hip ratio, diet, physical inactivity, diabetes mellitus, alcohol consumption, cardiac causes and apolipoprotein B to A1 while only ethnicity, sex, age and genetics have been identified as non-modifiable risk factors.[2] Among those, the prevalence of many risk factors such as obesity, diabetes mellitus type 2 or physical inactivity has increased over the last decades highlighting the need for stroke prevention, protection and recovery interventions.[3–5]

In the aftermath of a cerebral infarction a diverse range of haemodynamic, metabolic and ionic changes occur leading to necrosis and apoptosis of neuronal cells.[6] Core infarcted regions are surrounded by tissue at risk, the penumbra in which hypoperfusion leads to cytotoxic processes and depolarizations what then might eventually turn into infarcted tissue. Timely reperfusion of the occluded vessel either pharmacologically via thrombolysis or mechanically via thrombectomy are the only FDA and EMA approved interventions for the acute treatment of cerebral strokes so far.[7]

Symptomatology of cerebral infarctions differ depending on the cortical region affected. The most frequent observed symptoms are cognitive impairments, ataxia, hemiparesis, sensory impairments and aphasia among others. Stroke survivors regard impairments in cognition, but also aphasia, fatigue and exercise interventions to improve deconditioning as the most important areas of research to counter stroke symptoms.[8] Currently, it is expected that the neurological recovery process of cerebral strokes are limited to the first weeks after stroke but might extend up to six months.[9,10] Some improvements can still be achieved at a later stage using compensation techniques and acquisition of new skills. Coming from preclinical research, the evidence on changes in the brain during the acute phase is relatively robust.[6] Similarly, as many acute houses and stroke units are affiliated to university medical departments, the evidence for the acute phase has gotten stronger over the last decades. As a result, acute interventions such as mechanical thrombectomy or mobile stroke units – special ambulances with a CT-scanner and a specialized team on board – could prove its effectiveness in

decreasing stroke burden. On the contrary, there are high uncertainties towards the recovery processes and the concept of spontaneous recovery during the early and late subacute phases of stroke and the time point when true recovery ends.[9,11,12] As no pharmacological treatment was found to improve stroke recovery so far, the role of non-pharmacological interventions in multidisciplinary rehabilitation clinics is paramount. One of the main goals of inpatient rehabilitation is prevention of secondary events. This includes management of associated risk factors with dual antiplatelet therapy, oral anticoagulation in cases of cardiac origin, sustained lowering of blood pressure, lowering of LDL cholesterol concentration with statin therapy, blood glucose management, carotid revascularization if needed, as well as life-style changes such as smoking cessation, reduction in alcohol consumption, monitor glucose intake (low risk diet) and reducing sedentary time while simultaneously increasing physical activity.[12] One detrimental consequence of stroke that became more and more apparent over the last decades is that stroke survivors also suffer from deconditioning after long hospitalization periods.[13] Detrimental effects of sedentary behavior in healthy subjects alone include a decreased lipoprotein lipase activity or the uptake of triglycerides into muscle tissue, but also a decrease in maximal oxygen consumption.[4,14] Stroke survivors soon after stroke present with a decreased pulmonary capacity and an increased cost of walking.[15] The reduced initial aerobic capacity and the subsequent deconditioning might lead to a further decrease of physical activity after return to home.

One promising treatment to facilitate functional recovery after stroke is a structured aerobic exercise intervention. Aerobic exercise incorporates the movement of large muscles over a longer period of time with an increased heart and breathing rate.[16] Aerobic exercise has not only been found to increase maximum oxygen consumption in healthy volunteers[4] and stroke survivors[17] and thus counteract decreased endurance but also to lower gait energy cost, and to increase maximum walking speed in stroke patients.[18] Due to its effect on cardiovascular risk factors such as lowering of fasting insulin[19] or improving cerebral vasomotor reactivity[20], it became an important part of secondary stroke prevention.[21] Based on these results, aerobic exercise is a recommended treatment in many stroke rehabilitation guidelines worldwide.[21–23] Still, there is no detailed guidance on when the best time to start aerobic exercise is in stroke survivors. Cardiorespiratory exercise can be achieved with gait or treadmill training, training on a cycle ergometer or recumbent stepper, or aquatic training. Aerobic gait training is advantageous to improve mobility in stroke patients as it uses repetitive

locomotor movements which has been shown to improve walking and capabilities to perform activities of daily living in stroke patients.[24,25]

Additionally, it is hypothesized that aerobic training can improve outcome by enhancing endogenous neural plasticity, particularly if commenced early after stroke.[26] Studies conducted in rodents identified a facilitating effect of forced aerobic exercise on neurotrophic and neuronal growth factors such as brain derived neurotrophic factor, insulin-like growth factor-1 and nerve growth factor.[27] Also, an effect on lesion core and the penumbra has been observed in preclinical models.[28] Clinical evidence of aerobic exercise on neuroplasticity in the subacute phase of stroke depends on indirect measures. Changes in brain morphology or serum biomarkers such as brain derived neurotrophic factor, insulin-like growth factor-1 and vascular endothelial growth factor have been investigated in healthy volunteers[29] and stroke survivors.[30] In particular, changes in morphology and function of the hippocampus – a brain region sensitive for neuroplasticity, has been observed after aerobic exercise in healthy volunteers.[31] Furthermore, effects of aerobic training on the brain vasculature have been described in animal models of stroke.[32,33] Therefore it is hypothesized that aerobic training might improve stroke recovery if administered in the early phase after stroke.[34]

The evidence on aerobic training in subacute stroke patients is limited. Until the registration of the current dissertation a Cochrane review on Fitness training interventions in stroke from 2012[17] identified only two studies which applied a cardiorespiratory gait training early after disabling stroke but excluded studies with bodyweight supported training.[35,36] Aerobic treadmill training with bodyweight support enables exercising of moderately to severely affected, non-ambulatory patients. Patients with severe impairments seem to benefit the least from spontaneous recovery if proportional recovery rules are considered while having the highest need for improvement.[11] A systematic search of the literature in pubmed and hand searches of reference lists in related reviews including also bodyweight supported gait training revealed another four randomized, controlled trials (see search strategy in appendix 1).[37–40] Overall, results of previous studies are controversial with two studies showing higher improvements in maximal walking speed in the aerobic training group and two studies without significant differences between groups. Still, results derived from small studies with varying degrees of quality rendering comparison of results difficult. For

example, only one study reported training intensity.[36] Similarly, not all studies included patients with moderate to severe strokes.

In addition to the unclear evidence on the effects of an early aerobic training, feasibility of such a training regimen in the critical early post stroke phase remains unclear. No evidence was found for an increased risk in stroke patients so far but reporting of adverse events in these studies is poor and better reporting in cardiorespiratory training intervention trials is urgently needed.[17] Of the above mentioned studies one third did not mention adverse events at all while one third reported only flow of included patients and did not go into detail for dropouts during the study period. But especially in patients with moderate to severe strokes determinants of increased risks for SAE are needed to specify risk profiles for individual patients.

In response to the need to address one of the designated top ten research priorities in stroke rehabilitation[8] the Center for Stroke Research Berlin designed the 'Physical Fitness Training in Subacute Stroke – PHYS-STROKE' trial within the network of the Berliner Schlaganfall Allianz – a network of acute houses, rehabilitation wards and therapy centers in the Berlin area. The aim of my dissertation was to investigate the benefits and harms of a non-pharmaceutical therapeutic intervention in a medically unstable condition. More specifically, my goals were 1) to determine the efficacy of aerobic gait training on functional recovery and neuro-restoration as well as the feasibility of such a training compared to a non-cardiorespiratory active control intervention in the early subacute phase after stroke, and 2) to delineate carefully the characteristics of patients at higher risks of adverse events.

3. Methods

3.1 Study Design and participants

To answer this research question the multicenter, randomized controlled, endpoint-blinded PHYS-STROKE trial was conducted in seven rehabilitation clinics in the Berlin area, Germany. The trial was approved by the local ethics committee (EA1/138/13). A protocol has been published previously by the investigator group.[41] In addition, a statistical analysis plan including analyses from my dissertation has been detailed by the principal investigator, the lead biostatistician and me (https://doi.org/10.6084/m9.figshare.5375026.v1). In- and exclusion criteria as well as the screening process are detailed in publication 1. The following sections focus on methods not described in the included publications of my dissertation as well as on giving further background on methodological reasoning.

3.2 Intervention

Participants were randomized to receive either an aerobic gait training or relaxation therapy in addition to standard care. Both intervention arms were delivered for four weeks, with five sessions per week and each session lasting 50 minutes comprising 25 minutes of core intervention time. In the bodyweight supported, aerobic gait training group participants were trained either on a mechanical gait trainer (Functional Ambulatory Category (FAC) ≤ 2; Gait Trainer GT1; Reha-Stim, Berlin, Germany) or on a treadmill (FAC > 2; Multi-disk treadmill Callis, Model Therapie; Sprintex Trainingsgeräte, Kleines Wiesental, Germany; Reha-Stim, Berlin, Germany), dependent on functional mobility. For safety reasons, participants wore a parachute harness which could also be used to support bodyweight in cases of low postural control. Participants were targeted to train at 50 – 60% of maximum heart rate which was calculated by the pragmatic formula: Target heart rate = 180 minus years of age. In cases of β blocker intake target heart rate was reduced by 10 beats per minute.

During relaxation sessions, participants received progressive muscle relaxation focusing on the upper extremities, chest, shoulders, neck and face.

3.2.1 Methodological considerations

Non-pharmacological trials of sufficient size and quality to produce the highest level of evidence are lacking in stroke rehabilitation in Germany and worldwide. Although there might be different reasons underlying this phenomena, as a result most rehabilitation clinics are not prepared for larger randomized trials. Therefore, some considerations had to be taken in the beginning.

Several methodological decisions in the protocol followed a pragmatic approach. First, training of respective centers had to be done over several days but it was also expected that constant feedback with the study team at each site was needed. Many stroke rehabilitation clinics lack dedicated research staff. Although training was administered and a webpage with FAQs as well as a newsletter was created, variations in outcome assessment was expected if conducted locally by the involved therapists. As a consequence, two trained therapists did all assessments at all centers. Harmonization of outcome assessment was one benefit with the drawback of a demanding logistical effort.

Second, a query prior to the start of the trial lead to the understanding that most clinics were neither staffed nor equipped to conduct an exercise stress test with every included participant. That way, a decision was made that the treating therapist or physician would rate ability to perform aerobic exercise. Equally, a pragmatic formula which was easy to calculate was chosen to estimate the individual training heart rate. Here, the goal was to provide a way to easy administer the same training regimen during usual clinical practice while approximately assuring training at the aerobic-anaerobic threshold. The downside of this approach was that the individual differences in training capacity could not be accounted for. Still, we expected a close estimate of the true threshold.

Third, controlling heart rate during exercise of stroke patients is challenging. Usually, heart rate can be increased by increasing the speed of the device. Patients with severe impairments operationalized with a FAC Score of ≤ 2 were trained in a mechanical gait trainer. The stepping speed of this device was limited. One way to facilitate higher stress on the patients was to limit the respective bodyweight support leading also to higher stress on the musculature especially in the knee joints. We expected that patients with severe impairments would also be deconditioned and have a low aerobic-anaerobic threshold. Also, it was expected that due to the intervention and to spontaneous recovery mechanisms, many patients would quickly advance to the

treadmill. Starting with a FAC of ≥ 3 patients were planned to be trained on a treadmill with bodyweight support. Heart rate could be controlled by either increasing the speed of the treadmill, reducing the bodyweight support or increase the inclination of the treadmill. In many patients the possibilities to increase the speed was limited due to hemiparesis. In severe cases or patients with severe paresis of the peroneous muscle, therapists were instructed to assist setting the foot and helping to shift weight. Besides the patients capabilities to perform quicker movements the speed of the gait was also limited by the ability of the therapist to assist the movements. In such cases, therapists were instructed to increase the inclination of the treadmill to facilitate higher stress on the cardiopulmonary system if the target heart rate was not achieved. At higher inclination, stress on the knee joint was substantially increased which could limit progression as well. Especially in young participants, achievement of target heart rate was therefore challenging.

3.3 Outcomes

Outcomes were assessed at baseline, after the intervention as well as three and six months post stroke. As a co-primary endpoint, the change in maximal walking speed (assessed in m/s) and in the Barthel index at three months post stroke were chosen to reflect clinically meaningful improvements in gross motor function with high relevance for stroke patients.[8,42,43] Participants were allowed to use any orthosis used also during standard care physiotherapy. The Barthel index was assessed by our study assessors at each visit.

Exploratory secondary endpoints included maximal walking speed and Barthel index at post-intervention and at six months follow-up, walking endurance and as a physiological marker for effects on the cardiovascular system energy cost of gait at three months as assessed with the six-minute walk test. The six minute walk test was conducted in a 30m hallway with limited traffic but within the clinical setting of the respective study site. Each participant was asked to walk safe but as brisk as possible. At least one study assessor accompanied the participant to ensure safe commuting. Orthosis or assistive devices were allowed to be used. In an exploratory framework, gait energy cost was measured during the six minute walk test. Participants wore a mobile spirometry (k4B^2, Cosmed, Rome, Italy) which enables breath-by-breath analysis of oxygen consumption (VO^2), breathing frequency and heart rate during exercise with a high test-retest

reliability in stroke patients (Intraclass correlation in VO^2 of 0.9).[44] Study assessors were trained on the devices by Cosmed on a one day workshop and assessments regularly supervised by me. Devices were calibrated before each measurement following instructions from the company. Average VO^2 from the last three minutes of the six minute walk test was used and divided by body mass and meters to calculate gait energy cost per meter walked with the formula ml/kg-1/m-1. The secondary outcomes six minute walking distance and gait energy cost were chosen as they reflect the overall response of an endurance training and could be used to control whether training was delivered at an intensity to induce an effect on the cardiopulmonary system. To identify protective effects of the intervention on lesioned tissue as well as any association of lesion size on primary outcomes lesion sizes were assessed in ml in a subset of patients enrolled in the accompanying ‚Biomarkers and perfusion – training-induced changes after stroke (BAPTISe)‘ substudy of the PHYS-Stroke trial. Here, a sample of 110 patients received magnetic resonance imaging (MRI) at baseline and post intervention.[45] Only patients with data on both time points were used. Although patients were included early after stroke I did not expect any effects on the core lesioned area as effected areas die within the acute phase. It was hypothesized that neuroplastic effects and neovascularization might take place in the tissue surrounding the lesion and subsequently saving it from equally turning into dead tissue.

Safety endpoints were the serious adverse events (SAE) recurrent cerebrovascular or cardiovascular event, readmission to an acute care hospital or death within six months post stroke. SAE were reported by the study site physicians to the trial coordinating center. After discharge SAE were self-reported by the participants and confirmed by discharge letters. Health status of participants that were unavailable at the six months follow-up were retrieved from the population registry of Berlin. All adverse events were reported to a data safety monitoring board (DSMB) on a regular basis.

To identify risk factors for SAE in the early subacute phase after stroke we performed exploratory analyses on the association of SAE occurrence with clinical characteristics, pre-existing comorbidities, medication, and pertinent blood biomarkers assessed at baseline.

3.3.1 Methodological considerations

Maximal walking speed was measured on a hallway within the clinical setting. Participants had to walk 14 meters at their maximal tolerable walking speed of which

only the middle ten meters were analyzed. Times were taken with stop watches by our study assessors and the accuracy of measurements were controlled with laser beams in the beginning of the trial. Measurements with stop watches were accurate to the first decimal but measurements with the laser beams created measurement errors in patients with severe gait impairments which needed close support. Here, the foot of the assessor crossed the laser beam, resulting in inaccurate measurements. Therefore, the laser beams were only used in the first year of the trial to confirm measurements with stop watches.

Initially, the protocol aimed at assessing maximal oxygen consumption (VO_2 max) as a direct measure of physiological effects on the cardiopulmonary system. The measurement of VO_2 max would have required a maximal exercise test until fatigue. After assessing the possibilities to perform exercise testing at respective study sites it became obvious that this was not feasible in the course of the PHYS-Stroke trial. In addition, to date only one exercise testing procedure was validated for stroke patients.[46] In this protocol patients reached only a mean of 84% of maximal age-predicted heart rate, rendering it not suitable for a valid assessment of VO_2 max. Therefore, assessment of gait energy cost was considered a clinical meaningful measure resembling physiological changes. As outlined above, measurements with a spirometry needs precise calibration methods such as calibrating the valve and to compare the composition of the environmental air to a predefined calibration gas. Controlled conditions for humidity, temperature and air pressure are needed to precisely measure VO^2 during activities. Usually, measurements are conducted in laboratories. Such conditions were not to be expected in the clinical setting, so that a higher number of measurement errors had to be anticipated.

As outlined above, the DSMB overlooked all serious adverse events in a total of four meetings. During those meetings, adverse events were discussed with respect to the clinical history of the respective patients and the time point of the event. Even though a DSMB summary report on overall statistics was written by me (appendix 2), predefined analyses were not able to account for risk factors potentially related to any of the adverse events. Therefore, I designed the not-prespecified exploratory analyses detailed in publication 2.

As part of feasibility, treatment fidelity might need special attention in non-pharmaceutical interventions as along with the participants adherence, delivery of the intervention depends on the motivation and beliefs of the treating therapists and the

trust the therapist puts in the intervention protocol. Over the course of the trial, it became obvious that the perspectives of the therapists would need to be assessed to better understand feasibility of the training regimen in the context of a clinical setting. I therefore administered a self-designed questionnaire at the end of the trial in which I asked the therapists delivering either of the two interventions or both on their attitudes towards the intervention protocol. The questionnaire was not pre-specified. A link to the online questionnaire was sent out via e-mail to all therapists except for one study site in which access rights to the questionnaire was not granted and the questionnaire was delivered in a printed form. Participation was anonymous and included the following questions.

General questions:

What is your proficiency? Which intervention did you deliver? How much time was planned for each intervention session? How much time did one intervention session lasted on average?

Questions on the aerobic training:

Was a gait trainer available? Was a treadmill available? Was a bodyweight support available for the treadmill? How many therapists were available for caretaking of patients with severe impairments (FAC <2)? Did you had the impression that the patients trained at their aerobic-anaerobic threshold? Do you feel it is necessary to accurately determine the necessary training heart rate using an exercise test? Did the patients tolerate the training well? In your opinion, is aerobic treadmill training like the one performed well integrated into the daily clinical routine?

Questions on the relaxation training:

Do you have the impression that the patients have benefited from the training? If so, in what ways have patients benefited? In your opinion, was it possible for the patients to engage in the relaxation training? Did you have facilities available to conduct the relaxation training in an undisturbed environment? Did it happen that patients fell asleep during the intervention? If so, how often?

Questions on motivation:

Were your patients mostly motivated to do the intervention? Did the patient's motivation change during the course? If so, how? Were you motivated to lead or deliver the intervention? Has patient therapy progress helped you overcome your motivational problems? If no, how could the intervention be changed to make it more motivating for you?

Therapists were also given the chance to leave comments. Only descriptive and narrative summaries were planned for the data from the questionnaires.

3.4 Data analysis

MRI examinations were performed on a 3-Tesla MRI scanner (TIM Trio; Siemens AG, Erlangen, Germany) before and after the trial intervention. Sequences of the BAPTISe protocol used for the current analysis included a fluid attenuated inversion recovery (FLAIR) sequence (TE = 100 ms, TR = 8000 ms, TI = 2370.5 ms, field-of view [FOV] = 220 mm2, matrix = 256 × 232, 5-mm slice thickness with a 0.5-mm interslice gap, scan time 1:54 min) which was used to identify lesions and a T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE) structural image in the sagittal plane (TE 2.5 ms, TR 1900 ms, TI 900 ms, flip angle 9°, parallel imaging (GRAPPA 2), 1 mm isovoxel, scan time 4:26 min). Except for the design of the MRI sequences, the assessment of lesion size was designed by me and included the following steps: Lesion masking was conducted using a semi-automated pipeline. First, lesions were demarcated with the 'clusterize' toolbox following standard procedures[47,48] and are described shortly. Within 'clusterize', lesions were drawn on FLAIR images and wherever possible additional T1-weighted MPRAGE for subacute stages and diffusion weighted images were used for comparison on patients included in the late acute phases.

In a first step, hypo-intense areas were defined to better differentiate brain tissue from e.g. the ventricles using the reference T1-weighted MPRAGE structural images. The toolbox then created clusters of areas with corresponding grey values which were suspected to be a lesion. Subsequently, lesions were identified and manually selected under guidance of the clinical report from the acute care imaging. After all lesions were

masked, quality checks were done by a trained neuroradiologist with more than ten years of practice.

In a second step, lesion masks were co-registered to T1-weighted MPRAGE images using a customized pipeline. In detail, FLAIR images were co-registered with the Advanced Normalisation Tools (ANTs)[49] using cost-function masking and the registration matrix used to co-register the lesion mask to the T1 image. To account for differences in regional volumes of the brain due to age and sex differences intracranial volume (ICV) was calculated as the sum of grey matter and white matter and cerebrospinal fluid. Lesion volume was subsequently adjusted with the following formula: adjusted volume = raw volume – b * (ICV – mean ICV), with b denoting the slope of a regression of a region of interest on ICV.

3.5 Statistical analysis

Reported clinically meaningful differences of 0.13 m/s in maximal walking speed or 10 points in the Barthel index were used to inform our sample size calculation which resulted in 172 participants (86 participants per group).[50,51] For details please refer to publication 1.

Descriptive statistics are displayed with mean (SD) or median [IQR] where appropriate. Both endpoints were analyzed independently in two separate analyses of covariance (ANCOVA) within the full analysis set using all randomized participants. Dependent variables were the change in maximal walking speed or Barthel index at time point three months post stroke and baseline values and intervention group were the independent variables. All values missing at random (MAR) were imputed using multivariate imputation by chained equation (mice) with 10 imputed datasets and relevant information generated by the R package mice based on data used in the models.[52] Imputation is considered the best option to account for missing data and superior to other methods such as 'Last observation carried forward' in the field of stroke recovery research as patients characteristics change even without any intervention.[53] Subgroup analyses were calculated using interactions between treatment group and respective subgroups age, sex, stroke type, stroke severity operationalized with the National Institute of Stroke Scale (NIHSS), FAC and time from stroke onset to intervention start.[54]

SAE were analyzed using Poisson regression analyses with a random effect for center and adjusted for age, sex, and stroke severity as measured by the NIHSS. Poisson regression allows to calculate incidence rate ratios with confidence intervals for the observation period until six months post stroke.[55]

The exploratory risk factor analysis was equally analyzed by fitting multiple Poisson regression models to identify possible associations between comorbidity, treatment arm and occurrence of serious adverse events. To identify direction of interaction estimated marginal means were calculated.

To assess the effect of the intervention on lesion volume, an analysis of covariance was calculated with the change in lesion volume between pre and post intervention as dependent variable and respective baseline value as covariate. Analysis was adjusted for age, sex and FAC. Center was included as a random effect. An exploratory framework was used for lesion volume analyses. All exploratory analyses were not adjusted for multiple testing.

4. Results

4.1 Study population

During 2013 and 2017, 200 participants (3%) of initially screened 12 866 adults were randomized to receive either aerobic, bodyweight supported gait training (n = 105) or relaxation therapy (n = 95) in addition to standard care. In the beginning of the trial inclusion was not as high as expected and after two years it was decided to prolong the inclusion phase which was initially scheduled to only last for three years.

Table 1 | Baseline characteristics of the patients stratified by intervention

Characteristic	Training (n=105)	Relaxation (n=95)	All Patients (n=200)
Age in years, mean (SD), years	69 (12)	70 (11)	69 (12)
Female sex – n (%)	45 (43)	36 (38)	81 (41)
Time from stroke to intervention, median [IQR], days†	30 [17 – 39]	27 [17 – 41]	28 [17 – 40]
Anterior circulation stroke, n (%)	84 (80)	72 (76)	156 (78)
Hemiparesis on admission, n (%)	98 (93)	89 (94)	188 (94)
NIHSS score, median [IQR]‡	9 [5 – 12]	7 [5 – 11]	8 [5 – 12]
Ischemic Stroke, n (%)	91 (87)	90 (95)	181 (91)
Treatment with alteplase, n (%)§	34 (37)	27 (30)	61 (34)
Ischemic stroke aetiology§			
LAA, n (%)§	17 (19)	19 (21)	36 (20)
Cardioembolism, n (%)§	18 (20)	18 (20)	36 (20)
Small vessel occlusion, n (%)§	16 (18)	15 (17)	31 (17)
Other aetiology, n (%)§	3 (3)	4 (4)	7 (4)
Undetermined aetiology, n (%)§	34 (37)	28 (31)	62 (34)
Competing etiologies, n (%)§	3 (3)	6 (7)	9 (5)
Previous stroke – n (%)	27 (27)	27 (28)	54 (27)

† No data available in four patients, because patients were excluded as screening failures prior to intervention start. ‡ Scores on the National Institutes of Health Stroke Scale (NIHSS) range from 0 to 42, with higher scores indicating greater stroke severity. Assessed on day 3-5 after stroke. The NIHSS score of one patient was missing due to missing charts from the acute hospital. § Reported proportions of patients treated with alteplase and proportions of stroke aetiology refer only to ischemic stroke patients. LAA denotes large artery artherosclerosis. (modified after *Nave et al, 2019*)[56]

Baseline characteristics of included participants were mostly similar between intervention groups and are shown in table 1.

4.2 Efficacy

4.2.1 Primary efficacy endpoints

Results of the primary efficacy endpoint analysis are outlined in table 2 and reported in more detail in publication 1. In short, the adjusted treatment effect in maximal walking speed was 0.1 m/s (95% CI 0.0 to 0.2 m/s, p = 0.23). In the Barthel index score the intervention resulted in an adjusted treatment effect of 0 (95% CI -5 to 5, p = 0.99).

Table 2 | Primary efficacy endpoints

Co-Primary Outcomes (change from baseline to 3 months after stroke)				
	Training **(n=105)**	**Relaxation** **(n=95)**	**Treatment effect** **(95% CI)**	***P* Value**
Maximal walking speed, mean (95% CI), in m/s	0.4 (0.3 to 0.4)	0.3 (0.2 to 0.4)	0.1 (0.0 to 0.2)	0.23
Barthel-Index, mean (95% CI)	30 (24 to 36)	30 (23 to 36)	0 (-5 to 5)	0.99

Analyses based on multiple imputation. Treatment effects were analysed using ANCOVA mixed models with the three-month outcome as the dependent variable adjusted for baseline and additionally adjusted for sex, centre, and FAC. Values are presented as means with 95% confidence intervals (95% CI).(modified after *Nave et al, 2019*)[56]

Exploratory subgroup analyses could not identify a significant treatment effect in any of the subgroups except for sex with a group difference in maximal walking speed of 0.3 m/s (0.1 to 0.5) in women (see figure 2).
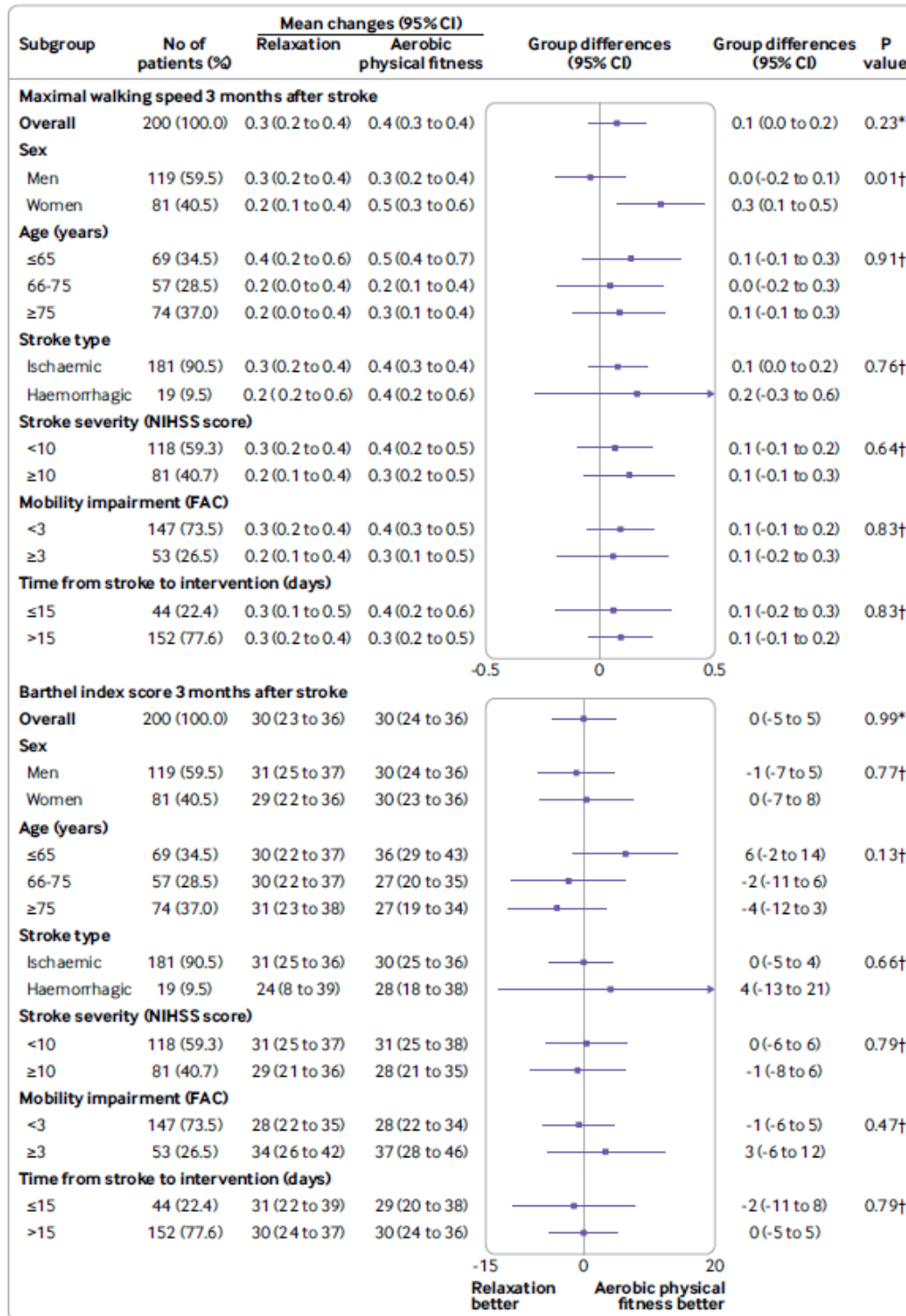
Subgroup	No of patients (%)	Mean changes (95% CI) Relaxation	Mean changes (95% CI) Aerobic physical fitness	Group differences (95% CI)	Group differences (95% CI)	P value
Maximal walking speed 3 months after stroke						
Overall	200 (100.0)	0.3 (0.2 to 0.4)	0.4 (0.3 to 0.4)		0.1 (0.0 to 0.2)	0.23*
Sex						
Men	119 (59.5)	0.3 (0.2 to 0.4)	0.3 (0.2 to 0.4)		0.0 (-0.2 to 0.1)	0.01†
Women	81 (40.5)	0.2 (0.1 to 0.4)	0.5 (0.3 to 0.6)		0.3 (0.1 to 0.5)	
Age (years)						
≤65	69 (34.5)	0.4 (0.2 to 0.6)	0.5 (0.4 to 0.7)		0.1 (-0.1 to 0.3)	0.91†
66-75	57 (28.5)	0.2 (0.0 to 0.4)	0.2 (0.1 to 0.4)		0.0 (-0.2 to 0.3)	
≥75	74 (37.0)	0.2 (0.0 to 0.4)	0.3 (0.1 to 0.4)		0.1 (-0.1 to 0.3)	
Stroke type						
Ischaemic	181 (90.5)	0.3 (0.2 to 0.4)	0.4 (0.3 to 0.4)		0.1 (0.0 to 0.2)	0.76†
Haemorrhagic	19 (9.5)	0.2 (0.2 to 0.6)	0.4 (0.2 to 0.6)		0.2 (-0.3 to 0.6)	
Stroke severity (NIHSS score)						
<10	118 (59.3)	0.3 (0.2 to 0.4)	0.4 (0.2 to 0.5)		0.1 (-0.1 to 0.2)	0.64†
≥10	81 (40.7)	0.2 (0.1 to 0.4)	0.3 (0.2 to 0.5)		0.1 (-0.1 to 0.3)	
Mobility impairment (FAC)						
<3	147 (73.5)	0.3 (0.2 to 0.4)	0.4 (0.3 to 0.5)		0.1 (-0.1 to 0.2)	0.83†
≥3	53 (26.5)	0.2 (0.1 to 0.4)	0.3 (0.1 to 0.5)		0.1 (-0.2 to 0.3)	
Time from stroke to intervention (days)						
≤15	44 (22.4)	0.3 (0.1 to 0.5)	0.4 (0.2 to 0.6)		0.1 (-0.2 to 0.3)	0.83†
>15	152 (77.6)	0.3 (0.2 to 0.4)	0.3 (0.2 to 0.5)		0.1 (-0.1 to 0.2)	
				-0.5 0 0.5		
Barthel index score 3 months after stroke						
Overall	200 (100.0)	30 (23 to 36)	30 (24 to 36)		0 (-5 to 5)	0.99*
Sex						
Men	119 (59.5)	31 (25 to 37)	30 (24 to 36)		-1 (-7 to 5)	0.77†
Women	81 (40.5)	29 (22 to 36)	30 (23 to 36)		0 (-7 to 8)	
Age (years)						
≤65	69 (34.5)	30 (22 to 37)	36 (29 to 43)		6 (-2 to 14)	0.13†
66-75	57 (28.5)	30 (22 to 37)	27 (20 to 35)		-2 (-11 to 6)	
≥75	74 (37.0)	31 (23 to 38)	27 (19 to 34)		-4 (-12 to 3)	
Stroke type						
Ischaemic	181 (90.5)	31 (25 to 36)	30 (25 to 36)		0 (-5 to 4)	0.66†
Haemorrhagic	19 (9.5)	24 (8 to 39)	28 (18 to 38)		4 (-13 to 21)	
Stroke severity (NIHSS score)						
<10	118 (59.3)	31 (25 to 37)	31 (25 to 38)		0 (-6 to 6)	0.79†
≥10	81 (40.7)	29 (21 to 36)	28 (21 to 35)		-1 (-8 to 6)	
Mobility impairment (FAC)						
<3	147 (73.5)	28 (22 to 35)	28 (22 to 34)		-1 (-6 to 5)	0.47†
≥3	53 (26.5)	34 (26 to 42)	37 (28 to 46)		3 (-6 to 12)	
Time from stroke to intervention (days)						
≤15	44 (22.4)	31 (22 to 39)	29 (20 to 38)		-2 (-11 to 8)	0.79†
>15	152 (77.6)	30 (24 to 37)	30 (24 to 36)		0 (-5 to 5)	
				-15 0 20 Relaxation better / Aerobic physical fitness better		

Figure 1 | Prespecified subgroup analyses.

Forest plots display maximal walking speed and Barthel index scores. Results are based on multiple imputation. No data were available for time from stroke to intervention for four participants who were excluded at screening. National Institutes of Health Stroke scale (NIHSS) score was missing for one participant owing to missing hospital chart. FAC=functional ambulation category. *P value for primary outcome measure. †P values for age×group interaction.(from *Nave et al, 2019*)[56]

4.2.2 Secondary endpoints

Exploratory assessment of gait energy cost during the six minute walk test captured only data of approximately 50% of the patients due to technical failures of the spirometry device, calibration errors within the clinical setting and non-plausible data. Of all assessed secondary endpoints, only the six minute walk test and gait energy cost showed a clinically meaningful difference between study groups of 27 m (95% CI 0 to 54) and -0.1 ml/kg-1/m-1 (95% CI -0.2 to 0.0) respectively at three months post stroke in favor of the training group. There were no substantial differences in maximal walking speed and Barthel index after the intervention or at six months post stroke. See secondary endpoints with treatment effects in table 3.

Table 3 | Secondary endpoints

	Group	Baseline Training n = 105 Relaxation n = 95 ¶	3 months follow-up Training n = 89 Relaxation n = 77 ¶	Treatment effect (95% CI)
6-minute walk distance, median [IQR], mean (SD), in m [b]	Training	75 [32 – 160] 107 (110)	165 [90 – 300] 201 (153) (n = 85)	27 (0 to 54)
	Relaxation	120 [39 – 205] 139 (113)	180 [110 – 263] 203 (128) (n = 71)	
Gait energy cost, median [IQR], in ml/kg-1/m-1 [i]	Training	0.8 [0.4 – 1.2] (n = 54)	0.4 [0.3 – 0.6] (n = 49)	-0.1 (-0.2 to 0.0)
	Relaxation	0.4 [0.3 – 0.7] (n = 46)	0.4 [0.3 – 0.6] (n = 41)	

Analyses are based on mixed models ANCOVA (adjustment for baseline value, age, sex, FAC, and center heterogeneity). Estimates are based on three level mixed models and multiple imputation (n=600 measures, 200 individuals, 6 centres) positive values favour aerobic training. Missing values were imputed by using multiple imputation. ¶ Number of available patients at scheduled visit. If number of valid data points differs in specific assessments number available is printed in the specific row. Data missing due to MAR are imputed as described in the appendix. § All secondary endpoints are exploratory and are not meant for hypothesis testing. *P*-values are thus not reported. [a] Seventeen patients could not walk for the entire time of six minutes. The distance they walked up to termination was used here. Twenty-eight patients could not do the test at all because of bad physical condition. For

those patients, values were imputed using single value imputation by taking half of the lowest value of the total cohort. [b] Spirometry data is missing mostly due to technical issues. Baseline values are available for 100 patients (54 Training, 46 Relaxation).(modified after *Nave et al, 2019*)[56]

Table 4 | Baseline characteristics of the convenient sample stratified by intervention

Characteristic	Training (n=44)	Relaxation (n=29)
Age in years, mean (SD), years	68 (12)	69 (11)
Female sex – n (%)	18 (41)	14 (48)
Time from stroke to first MRI, median [IQR], days	31 [17 – 35]	27 [17 – 39]
Anterior circulation stroke, n (%)	12 (27)	8 (28)
Hemiparesis on admission, n (%)	42 (96)	28 (97)
NIHSS score, median [IQR]	10 [8 – 12]	8 [6 – 11]
Lesion volume, median [IQR]	6.2 [1.6 – 41.3]	9.1 [2.3 – 21.4]
Treatment with alteplase, n (%)§	15 (34)	8 (28)
Ischemic stroke aetiology§		
LAA, n (%)§	7 (16)	3 (10)
Cardioembolism, n (%)§	4 (9)	3 (10)
Small vessel occlusion, n (%)§	11 (26)	8 (27)
Other aetiology, n (%)§	1 (2)	2 (7)
Undetermined aetiology, n (%)§	19 (44)	12 (41)
Competing etiologies, n (%)§	1 (2)	1 (3)
Previous stroke – n (%)	7 (16)	5 (17)

§ Reported proportions of patients treated with alteplase and proportions of stroke aetiology. One patient in training group had a hemorrhagic stroke. (Table created by: Rackoll)

Following the analysis of the primary efficacy endpoints in the full analysis set, I performed two separate analyses in a convenient sample of those participants that were also included in the BAPTISe study and had a pre and post intervention structural MRI. Seventy-two patients were included in the analysis. Baseline characteristics of the convenient sample resemble those of the full analysis set of PHYS-STROKE and are

shown in table 4. Lesion volume at baseline was positively correlated with NIHSS score three to five days after stroke (ρ = 0.57, 95% CI 0.39 to 0.71, df = 71) and negatively correlated with functional impairment at baseline measured with the FAC (ρ = - 0.35, 95% CI -0.53 to -0.35).

First, I performed a similar analysis as the one on the treatment effect on maximal walking speed at three months post stroke and added lesion volume at baseline as a covariate to assess whether size of the lesion was a confounder on the treatment effect. The overall treatment effect resembled the primary efficacy endpoint reported in publication 1 with 0.1 m/s (95% CI -0.1 – 0.3). Lesion volume at baseline did not confound the overall model.

Secondly, I analyzed whether the treatment had an effect on the lesion volume itself. Overall, treatment did influence the change in lesion volume between baseline and post-intervention with a difference in lesion volume change in the training group of -21.8 ml (95% CI -40.4 to -3.2). Further, I tested whether there was an interaction between sex and treatment as was observed in the subgroup analysis of publication 1. In contrast, an interaction of sex and treatment on lesion volume change could not be observed (-9.5 ml, 95% CI -47.2 to 28.1) as can also be seen in visualized model-based estimates using estimated marginal means in figure 3.
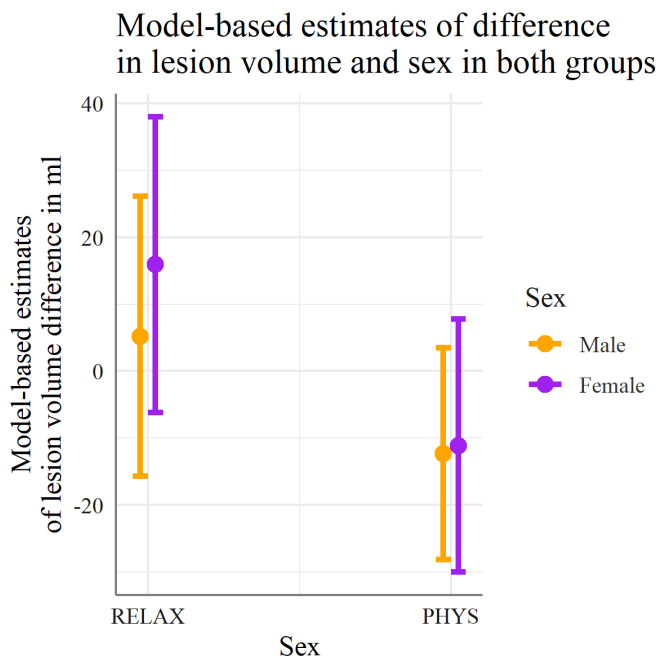
Figure 2 | Model-based estimates of lesion volume differences in ml per intervention group stratified by sex.

Estimates are derived from marginal means. (Figure created by: Rackoll)

4.3 Feasibility

4.3.1 Adherence

Participants started their intervention with a median time of 28 days [IQR 17 to 40 days] after stroke onset. In the aerobic training group, participants received a mean of 16 sessions (SD 6) with a training duration of on average 21 minutes (SD 4). In comparison, participants in the relaxation group received a mean of 17 sessions (SD 5) with a mean duration of 24 minutes (SD 3). During intervention sessions, participants of the training group were able to achieve the target heart rate in 70% [IQR 23 to 100] of sessions with better adherence towards the end of the intervention phase. Information on participant adherence is displayed in figure 3. At the end of each intervention session, participants were asked for perceived exertion using a ten-point visual analogue scale where 0 denotes not exhausting and ten highest exertion possible. Perceived exertion did not change in either of both intervention groups over the course of all sessions (Training: mean 5, SD 0; Relaxation mean 2, SD 0).
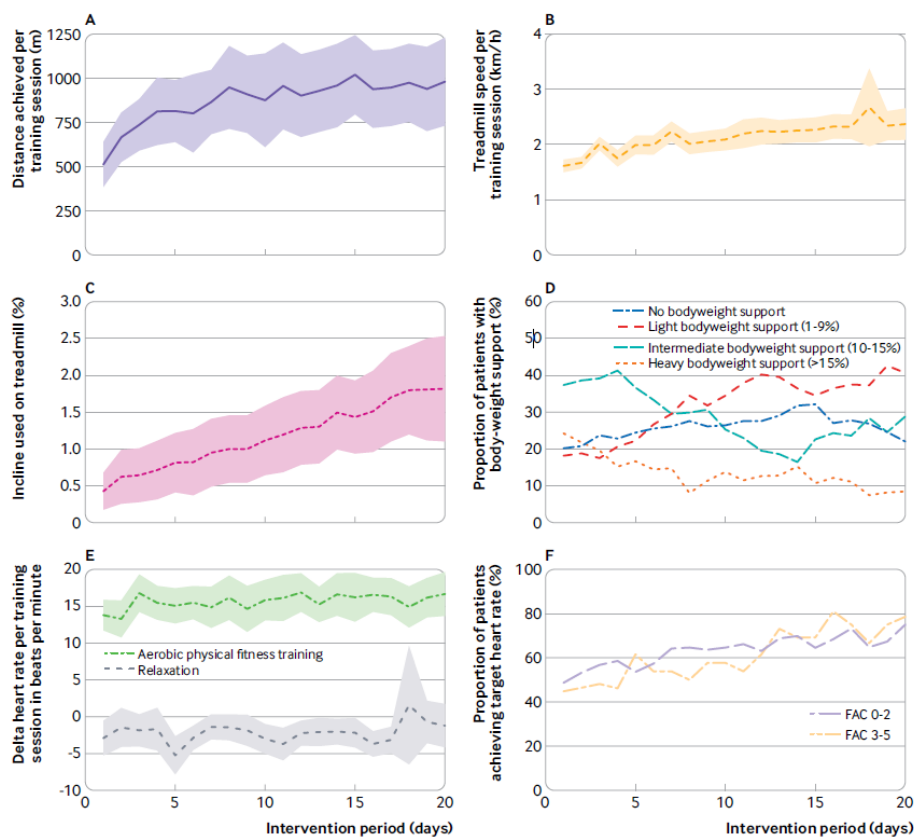
Figure 3 | Progression of training modalities during intervention period.

(A) Distance (m) achieved (only available for participants who used treadmill). (B) Walking speed (km/h) reached on treadmill. (C) Change in incline (%) on treadmill (only available for participants who used treadmill). (D) Proportion of participants with different levels of bodyweight support over time. (E) Mean change in heart rate by group measured before and after intervention sessions during intervention period. (F) Proportion of participants who achieved their target heart rate during training. Participants are grouped by baseline functional ambulation category (FAC) score of 0-2 and 3-5 (higher scores indicate less dependency).(from *Nave et al, 2019*)[56]

4.3.2 Training fidelity

The post-hoc questionnaire on treatment fidelity was answered by 19 therapists. Some therapists answered that they delivered both interventions. Sixteen participants answered questions concerning the aerobic training arm. Of those, 62.5% believed that the training was delivered at the aerobic-anaerobic threshold after the training heart rate was calculated with the pragmatic approach. Just as many (62.5%) thought it necessary that the target heart rate need to be assessed using exercise testing. All therapists had the impression that the patients did tolerate the training well. And 68.8% were convinced that an aerobic treadmill-based training can be integrated into clinical routine. In specific comments, therapists requested more detailed advise on when and how to adjust training intensity and that treadmill was in general better tolerated than the gait trainer.

Fifteen answers were given to questions focusing on relaxation therapy. Here, 60% were convinced that participants have benefited from the intervention and the others not. The majority (73.3%) of the therapists was convinced that the patients were able to engage with the relaxation activity.

Questions on motivation were directed on the perceived motivation of the patients and on the intrinsic motivation of the therapists to deliver the training. First, the therapists (100%) were convinced that the patients were motivated to participate in the intervention and the majority (73.7%) had the belief that this motivation did not change of the course of the intervention period while the rest of the therapists believed that it changed. The specific comments gave examples for both directions meaning that there were cases in which motivation declined as well as cases in which the motivation improved. Second, the majority of the therapists (89.5%) were motivated to deliver the intervention while the remaining were not (10.5%).

4.3.3 Adverse events

For primary safety endpoints, participants were followed up until six months post stroke. Ten participants were lost to follow-up (training, n = 4 *vs.* relaxation, n = 6). Until six months post stroke, 50 SAE were observed in 39 participants, of which 15 were recurrent cerebrovascular events, 30 were readmissions to an acute care unit and five participants died. The assigned data safety monitoring board rated all fatal events unrelated to the intervention.

Number and incidence rates from Poisson regression models are shown in table 4. Overall, associated risks for adverse events per 100 patient months were higher in the training group compared to relaxation group (IRR 1.70, 95% CI 0.96 to 3.12, *p* = 0.07) until six months post stroke. Only fatal events occurred less often in the training group (training, n = 1 *vs.* relaxation, n = 4, IRR 0.22, 95% CI 0.01 to 1.50, *p* = 0.18). Self-reported adverse events showed a higher associated risk per 100 patient months for falls in the training group compared to the relaxation group (training, n = 36 *vs.* relaxation, n = 14, IRR 2.34, 95% CI 1.26 to 4.34) during the intervention phase while risk for dizziness was higher in the relaxation group (training, n = 5 *vs.* relaxation, n = 14, IRR 0.33, 95% CI 0.12 to 0.90). All other self-reported adverse events did not show large differences between intervention groups.

4.3.4 Risk factor analysis

The post-hoc risk factor analysis identified higher associated risks for SAE in participants with diabetes mellitus and atrial fibrillation. The IRR for interaction of diabetes mellitus and treatment was 7.10 (95% CI 1.56 to 51.24) and for the interaction of atrial fibrillation and treatment was 4.37 (95% CI 0.94 to 31.81). Estimated marginal means for both models are shown in Figure 3a and b and the entire analysis are described in detail in publication 2.

Table 5 | Serious adverse events until six months post stroke

	All N=200	Training N=105	Relaxation N=95	IRR (Incidence- Rate-Ratio) (95% CI)	*p*
Follow up time (in days), median [IQR]	153 [139 – 164]	154 [140 – 166]	153 [135 – 163]		
Total SAE **IR 95% CI**	50 4.60 (1.99 – 8.53)	33 6.31 (2.89 – 10.82)	17 3.22 (0.87 – 6.91)	1.70 (0.96 – 3.12)	0.07
Cerebrovascular event **IR 95% CI**	15 1.58 (0.59 – 2.86)	11 2.25 (1.04 – 3.88)	4 0.93 (0.29 – 2.15)	2.43 (0.83 – 8.76)	0.13
Cardiovascular event	0	0	0	-	-
Readmission to hospital **IR 95% CI**	30 3.07 (1.45 – 5.08)	21 4.30 (2.23 – 6.69)	9 2.08 (1.00 – 3.75)	2.06 (0.97 – 4.73)	0.07
Death **IR 95% CI**	5 0.54 (0.09 – 1.17)	1 0.11 (0.00 – 0.89)	4 0.93 (0.19 – 2.15)	0.22 (0.01 – 1.50)	0.18

Incidence rates (per 100 patient-months) and Incidence Rate Ratios of serious adverse events between both intervention groups.(from *Rackoll et al, 2021*)[57]

a)

b)
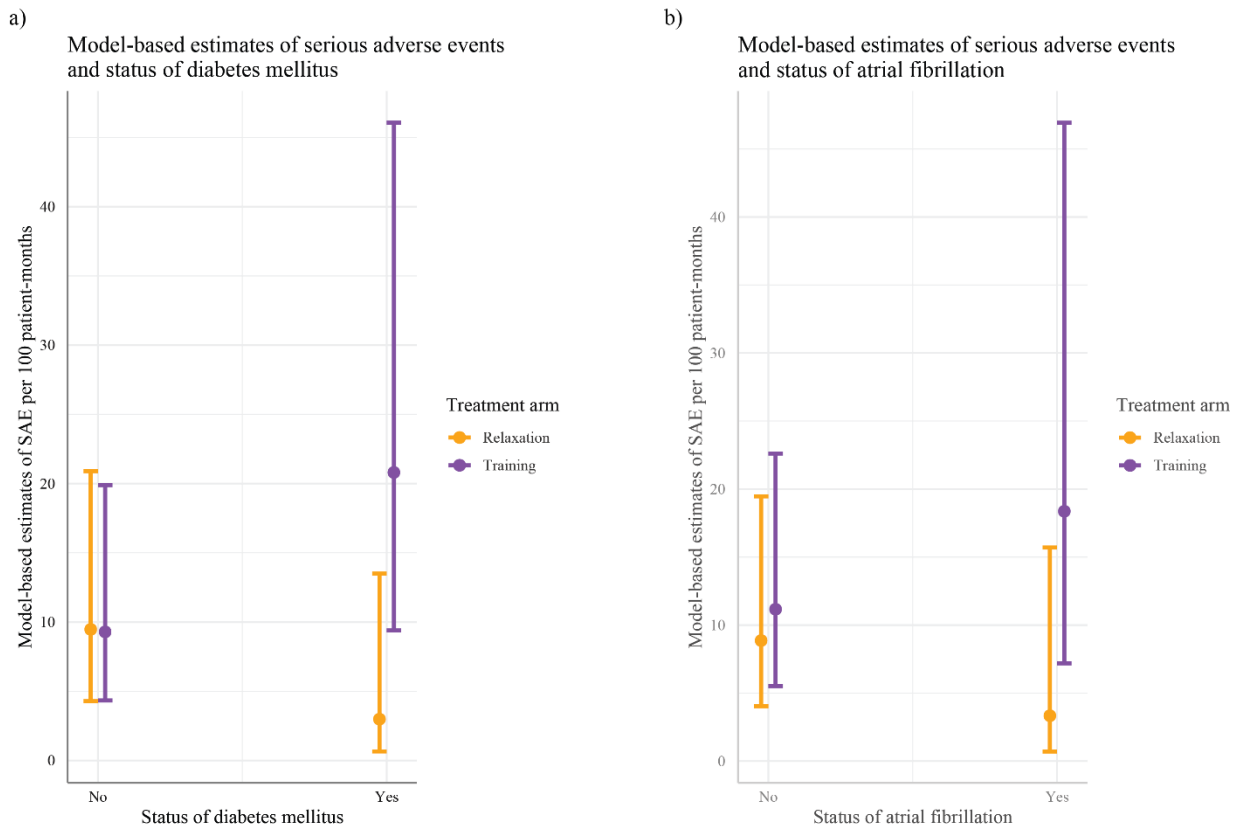
Figure 4 | Model-based estimates of events in subgroups of SAE for status of diabetes mellitus (panel A) and arial fibrillation (panel B) in both treatment groups per 100 patient-months.

Estimated marginal means are calculated from Poisson regression with Treatment, respective comorbidity, β-blocker medication and an interaction term for treatment with respective comorbidity and are adjusted for age, sex and NIHSS. Results are shown with 95% CI.(from *Rackoll et al, 2021*)[57]

5. Discussion

In my thesis, I examined the efficacy of an aerobic exercise training on mobility and activities of daily living compared to relaxation in subacute stroke patients and evaluated the feasibility of such an intervention. To summarize, our multicenter, randomized, endpoint-blinded trial provided grade 1 evidence that an aerobic, bodyweight supported gait training is not superior in moderate to severely affected stroke patients compared to a relaxation therapy on maximal walking speed and activities of daily living. Additionally, our trial recorded a higher number of serious adverse events and falls in the aerobic training group than in the relaxation group, resulting in an unfavorable risk profile for this patient group early after disabling stroke. The serious adverse events were mostly attributable to patients with concomitant diabetes mellitus and/or atrial fibrillation. In an exploratory framework, subgroup differences in sex on maximal walking speed, differential treatment effects on change in lesion volume as well as higher risks for serious adverse events after aerobic training in patients with pre-existing diabetes mellitus and atrial fibrillation were found.

5.1 Comparison with other studies: Efficacy endpoints

The results of the PHYS-STROKE trial are in line with the latest Cochrane Collaboration meta-analysis on physical fitness training in stroke as well as a recent systematic review and meta-analysis on physical fitness interventions in non-ambulatory stroke patients.[18,58] Effects seen in several trials on maximal walking distance measured with the six minute walk test were reproduced within our trial. No benefit was found in maximal walking speed and activities of daily living which contradicts findings from previous aerobic intervention trials in stroke patients.[36,59–63] Still, patients in these trials were either less severely affected or trained at a chronic stage. A moderately sized study which included non-ambulatory patients in the early subacute phase after stroke could equally not detect any beneficial effects on maximal walking speed.[37] Potentially, the dose delivered was too small to result in a clinically meaningful result. Based on current evidence, a clear dose response relationship cannot be discerned from the literature. While Mackay-Lyons et al. implemented the longest duration of an intervention phase with a total of 48 sessions over a twelve week period and could show an increase in the six minute walking distance but not in maximal walking speed, Klassen et al. did observe an intervention effect on both walking measures with a similar

protocol as ours but with a higher frequency of training sessions. Here, Klassen et al. applied two different doses in two cohorts, one with 30 minutes of aerobic exercise per weekday over a four week period and one dose with two 30 minute sessions per day of aerobic exercise every weekday. In this phase II, multicenter, randomized controlled trial the higher dose was found to be superior over usual care on maximal walking speed and walking distance. Improvements in walking endurance were also retained until the one-year follow-up assessment. Of note, our trial lacked a comparison with usual care control group so the additional effect on any intervention compared to no intervention cannot be determined. In our trial, adherence between both intervention groups as well as standard of care physiotherapy received were similar which makes it seem improbable that intervention groups received different doses of training.

In our trial, participants were trained on different devices depending on their functional impairment. Differences in the training modalities could have potentially influenced the results. Two recent Cochrane Collaboration meta-analyses analyzed the effect of treadmill training and electromechanical gait trainers on stroke recovery.[64,65] The first review found that treadmill training after stroke was not beneficial to gain independent walking but might help increase maximal walking speed and walking endurance. Benefits on walking speed and endurance were not clinically meaningful improvements and were contributed predominantly to independent walkers as suggested by a subgroup analysis. Our results could not show superiority of treadmill training but the majority of our study population was non-ambulatory. Of note, the authors of this review did not specify the definition of dependent walkers based on any scale therefore hampers comparison of results. In the second review, electromechanical gait trainers were found to be beneficial to increase maximal walking speed but not endurance. This is in contrast to our findings as our subgroup analysis could not reveal an effect on maximal walking speed in neither severely impaired nor less severely impaired patients operationalized with the Functional Ambulatory category. In our trial, patients with an FAC < 3 were trained on a gait trainer. However, quality of included studies in the meta-analysis was rated as low and the effect size of maximal walking speed did not reach a clinically meaningful improvement.

Within the last decade, preceding but also following our trial, sufficiently powered large multicenter trials were not able to replicate evidence from smaller trials within the field of stroke rehabilitation. Phase-III exercise intervention trials such as "A Very Early Rehabilitation Trial after stroke (AVERT)",[66] the "Locomotor Experience Applied Post

Stroke Trial" (LEAPS),[67] and the "Robot assisted training for the upper limb after stroke" (RATULS)[68] trial among others did similarly to our study not show superiority despite promising evidence. The PHYS-STROKE trial together with those other null result trials underpin the need for large confirmatory multicenter trials to pinpoint effectiveness of current or new therapies. Similarly, both AVERT and RATULS[69] raised the necessity for more evidence on dose-response relationships expanding the rationale for future phase-III trials.[70]

Sex differences in treatment efficacy has not been reported so far in any of the intervention trials included in any of the above discussed Cochrane Collaboration reviews. Evidence suggests differences in stroke outcomes and treatments but precise biological mechanisms have not been identified in preclinical research.[71] Also, the found difference could be related to other factors such as differences in behavior or fitness level prior to stroke. Lastly, as this was a finding within one of our subgroups it can also be due to chance alone. Future trials should follow up on the sex differences seen in the exploratory (pre-specified) subgroup analyses. Moreover, they should include assessment of pre-stroke physical fitness level and more behavioral data to better explain possible sex differences.

In the post-hoc analyses of treatment effects on changes in lesion volume post intervention, lesion volume was reduced in the training group compared to relaxation. Neuroprotective effects on lesion volume have been observed in animal research following forced exercise interventions. Such effects were found in the early subacute phase up to 28 days following induction of stroke. Reported effects were larger if exercise commenced between 24 and 48 hours after stroke with little effect seen with commencement after 7 days. In clinical research, neither of the aerobic exercise intervention trials with subacute stroke patients assessed effects on lesion volume. Following cerebral ischemia, lesion core transforms to dead tissue over the first days but is not considered to prolong much after 72 hours after stroke.[6] Only the surrounding penumbra can consequently be targeted by any protective intervention delivered after 72 hours. Although the surrounding tissue is at risk for a longer period, it seems rather unlikely that any effect will be seen more than 28 days after stroke onset which was the median time between stroke onset and start of intervention in the PHYS-STROKE trial. Still, critical time windows for restorative outcomes seem to be different between rodents and humans. The recently published phase II "Critical Period After Stroke Study" (CPASS) found most improvements on functional outcome one year after stroke,

if intensive training was delivered between two and three months after stroke onset which is much later than most effective time windows in rodents.[72]

Within the PHYS-STROKE cohort, no intervention effect was found on the blood biomarkers high-sensitive C-reactive protein, interleukin-6, tumor necrosis factor-alpha and fibrinogen as analyzed by Kirzinger et al.[73] It could be possible that the exercise intervention had an effect on the healthy tissue surrounding the lesion. Here, neuroplastic or neurovascular remodeling could have occurred which might increase the volume of the surrounding tissue and subsequently squeezing the dead lesioned tissue and thus minimizing its volume. As part of the BAPTISe trial, together with Kufner et al we investigated the moderating effect of the intervention on vessel size and vessel density pre and post intervention through vessel size imaging as well as their change over time and could not identify any treatment effect.[74] A neurovascular effect in the surrounding tissue or any protective effect on markers of inflammation therefore seems unlikely. Based on the current analyses and evidence from the literature, I would therefore conclude that the found effect is more likely to be due to chance and needs to be investigated further.

5.2 Comparison with other studies: Safety endpoints

Participants in our study showed a higher number of serious adverse events reported so far in any comparable exercise intervention trial. Neither of the referenced meta-analyses on exercise interventions in stroke patients found an increased risk for adverse events.[18,58,64] Also, a meta-analysis on adverse effects of exercise therapies comprising a large number of patients in several clinical populations did also not find a higher risk of SAE for patients in training groups.[75] Exercise interventions were therefore considered safe within the stroke population. Still, all meta-analyses critized inconsistent and incomplete reporting of adverse events. Also AVERT and LEAPS did similarly to our study reported a higher number of adverse events in the experimental intervention group.[66,67] In our trial we particularly found a higher number of recurrent strokes in the aerobic training group compared to none in the relaxation group during the intervention period. The early subacute phase after stroke is vulnerable for complications.[76] Within the substudy BAPTISe, Müller et al found increased blood-brain barrier permeability in 75% of the patients illustrating the vulnerability of the brain up to the start of the intervention phase.[77]

Information on risk factors associated with adverse events in exercise interventions after stroke are limited. Until now, associations of comorbidities and other stroke related factors to adverse events in exercise intervention trials are not discussed in the literature. Some studies reported most frequent comorbidities and medication, but no risk factor analyses were conducted.[39] Reporting of adverse events in exercise intervention trials was found to be poor. In the Cochrane Collaboration review on fitness training in stroke only seven out of 17 trials mentioned serious adverse events at all. Of those, only three studies actually reported the occurrence of any serious adverse event. In our risk factor analyses, we found a higher association of patients with pre-existing diabetes mellitus or atrial fibrillation with serious adverse events. Of note, risks for serious adverse events were comparable for patients without diabetes mellitus or atrial fibrillation in both intervention arms.

So far, risks of aerobic training for adverse events in stroke patients with diabetes mellitus were not investigated. Current guidelines for physical activity in diabetic patients recommend to closely monitor levels of glycemia to prevent patients from exercising during phases of acute hyper- or hypoglycemia.[78,79] A recent study found a lower cerebrovascular response to exercise in stroke patients with chronic hyperglycemia.[80] However, if there is indeed a relation between the presence of hyper- or hypoglycemia and higher risk of SAE in patients undergoing aerobic training after stroke has not been conclusively shown, and cannot be determined for certain in our exploratory analysis. A detailed assessment of glycemic control in stroke patients using aerobic training should be conducted in future trials.

Within our risk factor analysis we also found an associated higher risk of adverse events with aerobic training in patients with atrial fibrillation. In general, patients with atrial fibrillation demonstrate a high risk of stroke, and cardioembolic strokes tend to be larger compared to non-atrial fibrillation strokes.[81–83] Little is known about feasibility of aerobic training in the stroke population with concomitant atrial fibrillation. Aerobic exercise is an established and safe non-pharmacological treatment for patients with atrial fibrillation[84] but evidence from large trials on safety in this population is still not available.[85] High intensity aerobic exercise in patients with atrial fibrillation can be associated with cardiac distress.[86] Slow progression in training intensity is recommended in guidelines for early cardiac rehabilitation.[87] Subacute stroke patients with cardiac arrhythmias might benefit from similar slowly progressing training protocols.

Participants in the aerobic training group were at a higher risk for self-reported falls during the intervention period. Several other studies and meta-analyses observed an association of falls with exercise interventions in stroke patients.[67,88] In a subsequent risk factor analysis of the LEAPS trial, individuals with severe walking impairments were at higher risks for falls and balance impairments were found to be predictive.[89] The LEAPS trial classified patients with a gait speed of ≤ 0.4 m/s as severe walking impaired. The study population in our trial corresponds to this population. Therefore, our findings underpins the exposure to fall related adverse events in this population. In our trial we did not assess balance skills as the amount of assessments were already large and reasons for falls are multifactorial. Self-reported dizziness was found to be higher in the relaxation group. So far, no other exercise intervention trial reported this finding. Based on our data, higher self-reported dizziness in the relaxation group cannot be explained and need further assessment in future trials.

5.3 Comparison with other studies: Training adherence and fidelity

None of the studies applying aerobic treadmill based training in the subacute phase assessed fidelity and only some reported measures of training adherence.[36,59,60,63] The PHYS-STROKE trial was not designed as a feasibility trial but measures were taken to assess how patients and therapists perceived the training intervention, how the training was delivered and how much the patients adhered to the intervention protocol. Currently, there is no strict consensus on how and what to assess in terms of treatment adherence and fidelity in stroke studies. An early assessment of treatment fidelity within the "Exercise Training for Hemiparetic Stroke Intervention Development Study" (The Treadmill Study) by Resnick et al applied a treatment implementation model by Lichtstein, Riedel and Grieve to address 'delivery', 'receipt' and 'enactment' of the treatment which were complimented with the domains 'design' and 'training'.[90,91] Design refers to the overall concept of the experimental and the control intervention, that they were different in the mode of exercise that was addressed and that no carryover between intervention arms occurred. While the Treadmill Study had difficulties in achieving fidelity as participants travelled together to the intervention site and trained together, participants of the PHYS-STROKE trial were not able to train independently within the inpatient setting. In addition, several clinics included only one patient at a time which prevented patients to verbally discuss contents or experiences of either of the

intervention arms. The domain 'training' refers to the training of the staff delivering the treatment. Investigators of The Treadmill Study were concerned of a drift in intervention fidelity due to a change in staff over the course of the five year duration of the study. Here, exercise physiologists were trained in the beginning of the study and further training was conducted onsite using a "see one, do one, teach one" model and accompanied by regular meetings with the principal investigators. While this is a preventive measure, no investigations were conducted to quantify any possible drift from the exercise protocol. With the questionnaire this was in part addressed within PHYS-STROKE. Motivation was high among the therapists and also among the patients as reported by the therapists. Still, therapists mostly would have preferred exercise testing to determine the required training heart rate and some requested further training on how to modulate training intensity. Therefore some drift might have occurred throughout the study which might have been prevented by even more or more detailed supervision. 'Delivery' addresses the amount of training that was actually delivered including the amount of time participants trained at the target heart rate. Within the first three months of The Treadmill Study participants trained for a similar duration with a median of 23 minutes. Also, in this study only 48% of the participants reached the targeted 60 to 70% of maximum heart rate until the end of the study even though intervention period lasted over a six months period. A similar response in slowly progressing to targeted training intensity has been expected and can also be seen in other subacute stroke cohorts.[36,60] Of note, the effect seen in the secondary endpoints six minute walking distance and gait energy cost indicate that training was actually administered at an intensity that induced a training effect. 'Receipt' focuses on how the participant perceived the intervention, acquired new information and learned new behavior. In the outpatient setting of The Treadmill Study, coming to the intervention site was used as a proxy to address receipt of the intervention. Here, a qualitative assessment would be better suited within the clinical setting in which therapies are scheduled. Similar to PHYS-STROKE no assessment of patients perception beyond rating of exhaustion were performed. Lastly, 'enactment' refers to the behavioral change that might occur after the intervention period in the home setting and was neither addressed in this study nor in PHYS-STROKE. A more qualitative approach was used in the "Community-based rehabilitation training after stroke" (ReTrain) trial, in which interviews were conducted with participants.[92]

In general, the influence of therapist but also patient motivation on training success is an understudied field in stroke rehabilitation. Although the PHYS-STROKE trial did not quantify bias through training fidelity, a first approach was undertaken to address one important aspect of exercise interventions in the clinical setting which should be more formalized and considered in upcoming reporting guidelines of exercise intervention trials.

5.4 Strength and limitations

This is the first large randomized controlled phase III trial examining efficacy and feasibility of aerobic gait training in patients with moderate to severe subacute stroke which will inform clinical practice and future guidelines. High quality stroke recovery research from large phase III trials providing grade 1 evidence are desperately needed within the clinical field.[93] With the results of this study, determinants for tolerability of aerobic gait training are provided which in the future might lead to more personalized training approaches in this patient group.

Several limitations have to be considered when interpreting our results. PHYS-STROKE included a large variety of patients with heterogeneous strokes at different time points post stroke. Variations in stroke severity and recovery might be too broad to capture significant improvements and might have been solved by using stricter inclusion criteria or fixed time points. Even though influence of high variability in our population cannot be ruled out, subgroup analyses on the influence of stroke severity or time from stroke to start of intervention could not identify heterogeneous results. Further, participants in the aerobic training group were more severely affected although computerized randomization was used. Influence of worse baseline values were accounted for in the statistical analysis and it should therefore not have been dominant in our study. PHYS-STROKE was not powered to detect differences in severe adverse events. Safety analyses are exploratory and should be investigated in future trials. Little information was gathered on pre-stroke activity and behavior which hampers analyses on risk factors, or on identification of responders to aerobic exercise. Pre-stroke behavioral information often relies on self-reports or information from relatives which are prone to recall bias. Still, future trials should incorporate rigorous assessment on such behavioral information. Assessment of training fidelity was not pre-specified and therefore not operationalized within the trial protocol. More rigorous assessment of training fidelity will

enhance judgement of risk of biases through e.g. modes of delivery. Still, PHYS-STROKE was the first large multicenter aerobic exercise intervention trial to rigorously report treatment fidelity.

5.5 Conclusions and future research

The current dissertation investigated the effect of an aerobic gait training on maximal walking speed and Barthel index but could find no superiority compared to a relaxation intervention. Additionally, a higher risk in patients after moderate to severe strokes was found which should be considered in clinical practice and future trials. Training fidelity and adherence was moderate to good. Exploratory findings on sex differences and effects on lesion volume should be further investigated in future phase II trials while systematic reviews with meta-analysis might help to clarify the found association of higher risk of adverse events in patients with diabetes mellitus or atrial fibrillation. To conclude, based on these results an aerobic gait training in this population of moderately to severely affected stroke patients cannot be generally recommended in the early subacute phase after stroke.

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7. Appendix

Appendix 1: Search strategy

Pubmed search terms:

1. "Cerebral Hemorrhage"[MeSH] (35,677)
2. "Intracranial Hemorrhages"[MeSH] (75,531)
3. "Stroke"[MeSH] (151,957)
4. "Brain Ischemia"[MeSH] (116,565)
5. "Brain Infarction"[MeSH] (40,147)
6. "Cerebral Infarction"[MeSH] (34,039)
7. 1 OR 2 OR 3 OR 4 OR 5 OR 6 (263,686)
8. Subacute (42,464)
9. Early (1,731,973)
10. 8 OR 9 (1,770,324)
11. 7 AND 10 (28,212)
12. "Exercise"[MeSH] (222,284)
13. "Exercise Therapy"[MeSH] (57,556)
14. "Physical Fitness"[MeSH] (33,837)
15. 12 OR 13 OR 14 (276,565)
16. Aerobic (553,811)
17. 15 AND 16 (263,388)
18. 11 AND 17 (576)
19. 18 (Filters: Clinical Trial, Randomized Controlled Trial) (233)
20. 19 (Filter: Date range 1999 – 2014) (130)

Appendix 2: Data safety monitoring board summary report

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CHARITÉ ○ CSB
UNIVERSITÄTSMEDIZIN BERLIN   Centrum für Schlaganfallforschung Berlin

Bundesministerium für Bildung und Forschung

Clinical Trials Identifiers: NCT01953549 & NCT01954797

Physical Activity in Subacute Stroke (PHYS-Stroke)

PHYSSTROKE

Biomarkers And Perfusion – Training-Induced changes after Stroke (BAPTISe)

Data Safety Monitoring Board

Summary Report

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Principal Investigator PHYS-Stroke: Agnes Flöel	Principal investigator BAPTISe: Martin Ebinger
Study coordinator PHYS-Stroke: Torsten Rackoll	Study coordinator BAPTISe: Alexander H. Nave

Reviewer:

_____   _____
Prof Dr. med D. Steube   PD Dr. med. G.J. Jungehülsing

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Abbrevations

AE	Adverse Events
ANCOVA	Analysis of covariance
ADL	Activities of daily living
BI	Barthel-Index
BMBF	Bundesministerium für Bildung und Forschung
BMI	Body mass index
CRF	Case Report Form
DSMB	Data Safety Monitoring Board
EVE	Einverständniserklärung (informed consent)
FAC	Functional Ambulation Category
GS	Gait Speed
HR_{max}	Maximum Heart Rate capacity
PHYS	Fitness training Intervention (experimental condition)
PP	Per Protocol
RELAX	Relaxation training intervention (control condition)
SAE	Serious Adverse Events
V1	Visit 1 (Post-Intervention)
V2	Visit 2 (3 months after index stroke)
V3	Visit 3 (6 months after index stroke)

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Report Summary

Background

Within the subacute phase of stroke aerobic exercise including treadmill training is a promising rehabilitative therapy to improve walking abilities and minimize physical deterioration.

Methods

In this trial 200 of 204 patients in the early subacute phase of stroke (day 5-45 after stroke) were recruited from seven rehabilitation clinics in and around Berlin. Patients were randomized to receive 25 minutes of either treadmill-based, aerobic physical exercise (study intervention) or relaxation sessions (control intervention) five times per week for four weeks, in addition to standard rehabilitative therapy. Safety endpoints were recorded until 6 months post stroke and comprised recurrent fatal or no-fatal cardiovascular event including stroke, hospital re-admissions, and death.

Results

Safety analyzes are on the basis of 200 patients who were randomized in the trial (end of recruitment: April 30th, 2017; Database closed: Nov 10th, 2017). Adverse events and incidence rate ratios with 95% confidence intervals are presented, as well as medical characterization of patients who experienced an event.

Conclusion

Even though higher rates of recurrent cerebrovascular events as well as referral to an acute house are present in the intervention groups neither of those reached significance and thus no change in risk evaluation needed to be undertaken. Higher self-reported falls reached statistical significance in the intervention group but did not lead to serious injuries.

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Introduction

Preface

Due to modern therapies an increasing number of stroke patients survive the initial stroke event. From all sequelae of a stroke, the most severe consequences for patients, their families, and the society are restrictions of walking ability and restrictions of activities of daily living such as dressing, self-care, and communication. Therapeutic approaches, such as physiotherapy or occupational therapy are often lengthy and not always successful. Therefore new therapeutic approaches are tested. A promising approach is a fitness-training which is possible even in patients with severe stroke due to body weight-supported treadmill training.

Study Objectives

The aim of the PHYS-STROKE[1] trial is to evaluate if a 4-week physical exercise fitness-intervention (PHYS: target intervention) compared to a relaxation intervention (RELAX: control intervention) results in a significantly higher gait speed (in m/s, 10m walk) as well as a better performance of activities of daily living (assessed by the BI) in subacute stroke patients. The underlying physiological changes induced by the interventions will be analyzed in BAPTISe[2], a prospective observational study accompanying PHYS-STROKE. The target and the control intervention are additional interventions to the standard rehabilitation program of each study center.

Purpose of the report

This report will summarize patients disposition to potential harms of the physical exercise training intervention in the Phys-Stroke trial and analyze the safety of such an intervention in general.

Trial Summary

General Study Design and Plan

PHYS-STROKE is a prospective randomised controlled trial. The target intervention is a physical fitness training. The control intervention is a relaxation programme. BAPTISe is a prospective observational study accompanying PHYS-STROKE.

Time schedule: The total time frame for this study is 4 years and 6 months. The time schedule is as follows:

- month 0-2:   presentation of study in study centers, training of raters,
- month 3-39:   recruitment of patients (30 months in total)

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- month 3-45:   baseline- and follow-up visits

- month 45-48:   statistical analysis and preparation of scientific publications
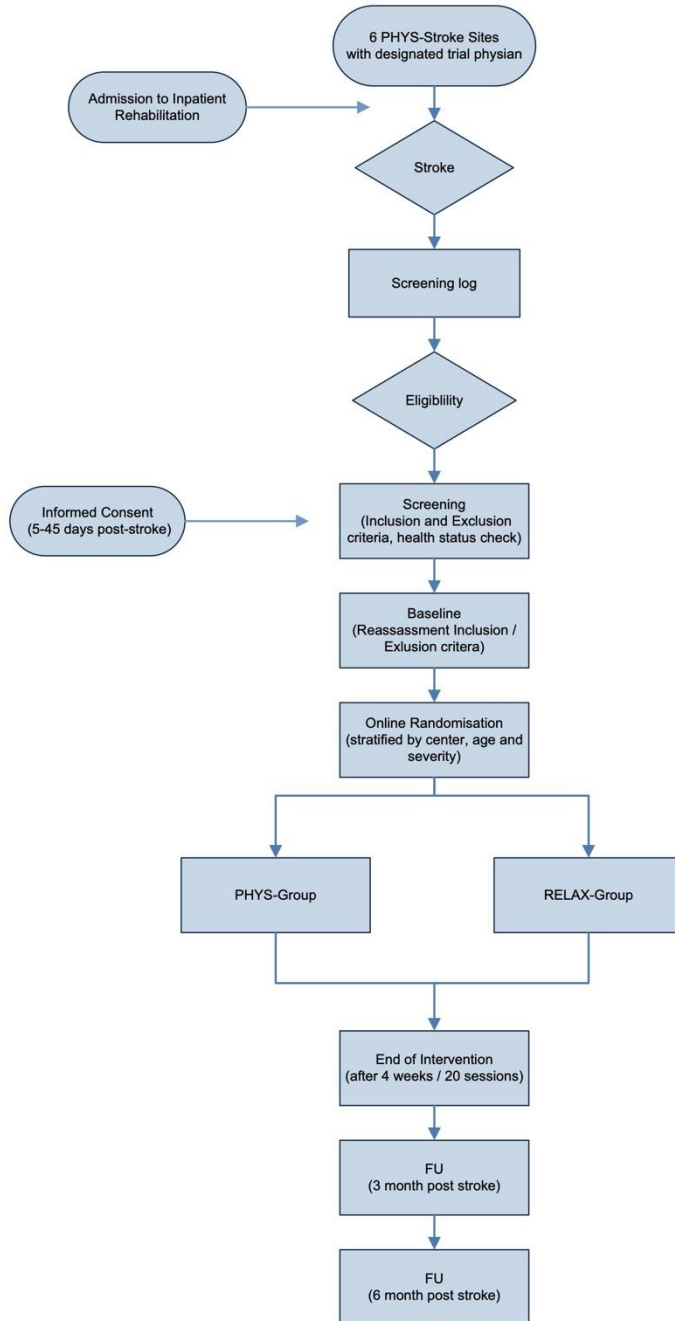
Study Course

Table 1: List of safety assessments and timing of assessment

	Screening	Baseline day 0-day 7 (or day 8-day 15*)	Intervention (next working day)	V1 (+1 Mo)	V2 (+3 Mo)	V3 (+6 Mo)
In-/Exclusion criteria	X					
Neurological and medical examination	X					
Randomisation		X				
4 week intervention, daily documentation			X			
Documentation of neurological status, grade of disability, medications, type of stroke etc.		X				
Systolic/diastolic blood pressure		X	X	X	X	X
Heart rate		X	X	X	X	X
MRI incl. vascularization and blood sample (optional; BAPTISe study)		X		X		
Safety (SAEs)		X	X	X	X	X
Safety (AEs)			X			

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Figure 1: Study Course

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Summary of Changes to the protocol

In the course of the study four amendments (02.09.2013, 23.04.2015, 15.11.2016 & 21.08.2017) were approved by the ethics committee of the Charite Universitätsmedizin Berlin. The following data was added:

Change of inclusion criteria 3) "Independent sitting possible (without external support) for 30 sec." to "Sitting for at least 30 sec possible (with or without support)"	Amendment 1 (Votum 02.09.2013)
7 day accelerometry and Freiburg questionnaire on physical activity (short version)	Amendment 2 (Votum 23.04.2015)
Audio recording of the Regensburg semantic and phonemic word fluency test	Amendment 3 (Votum 15.11.2016)
Small administrative changes (no additional assessments)	Amendment 4 (Votum 12.09.2017)

Summary of study definitions

Subject disposition

Definitions

Screening Failure: A patient who withdraws or is withdrawn from the study for any reason before receiving first day of intervention will be considered a screening failure. For reporting, screening failures are divided between 'being randomized' and 'not yet randomized'. Screening Failures are neither included in the full analysis set, nor in the PP set.

Withdrawal from treatment: A patient who received less than 75% of the intended intervention treatments for any reasons or missed intervention on more than five consecutive days (see 6.4) will be considered a 'withdrawal from treatment'. If possible V2 measurements will be acquired. Those subjects will be included in the full analysis set.

Lost-to-follow-up: Patients who did not receive a V2 assessment for any reason will be considered 'Lost-to-follow-up'. Those subjects will be included in the full analysis set.

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Protocol deviations

Inclusion – and exclusion criteria: Patients not meeting the exclusion criteria will be considered a screening failure and will therefore be excluded from analyses. Patients will still be included if minor violations of inclusion criteria are found such as:

- no imaging is available confirming the stroke but obvious signs of stroke are present

- subjects included earlier than 5 days post stroke, if the intervention did not start before the fifth day post stroke

- subjects included after 45 days post stroke, if the intervention started within 55 days post stroke

Patients with minor violations will be analyzed according to the allocated analysis set (full analysis set vs. PP set). To ensure that the pre-defined minor protocol violations do not influence the main findings, the PP set will be additionally analyzed without the protocol violators (sensitivity analysis).

Treatment Compliance

Intervention: Patients were required to receive at least 75 % days of intervention. If a patient is not able to receive the intervention on a specific day, the intervention day is added after the last intervention day if possible. Additionally patients are allowed to miss training only up to five consecutive days.

Serious Adverse events and adverse events

Safety parameters (severe adverse events): monitored continuously throughout the trial, and recorded at baseline, end of intervention , 3 month and 6 months post stroke:

- recurrent fatal or non-fatal cardiovascular or cerebrovascular events

- referral to an acute hospital

- death.

Safety parameters (adverse events): self-reported events assessed after each session during intervention time:

- pain

- fatigue

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- dizziness

- number and nature of falls

- other.

Safety analyses

For each assessment (baseline, end of intervention, 3 and 6 months post-stroke), the following parameters will be reported separately as incidences (n, %) in total and by intervention group: recurrent fatal or non-fatal cardiovascular or cerebrovascular events; referral to an acute hospital; death. Additionally for each intervention, reports of number of patients with the presence of self-reported pain, fatigue, dizziness, falls, and note other adverse events will be given in total and by intervention group. The safety analysis will be done in the full analysis population. When calculating the incidence of adverse events, or any sub-classification thereof by treatment, time period, etc., each subject will only be counted once and any repetitions will be ignored; the denominator will be the total population size. Group differences will be tested at different time points using Fisher's exact test. Additionally intervention documentation will be analyzed for heart rate and blood pressure response, fatigue, and self-reported exhaustion (via a visual analog scale).

Summary of DSMB meetings

The DSMB met four times in the course of the Phys-Stroke trial (23.06.2015, 24.05.2016, 18.04.2017 & 14.11.2017). Occurrences of adverse events were discussed as defined in the study protocol. Following cases were discussed:

Table 2: Summary list of DSMB meetings

DSMB Meeting	No. of Cases	No. of SAEs	Commentaries
DSMB 1 (23.06.2015)	50	7	No influence of the intervention was seen in the presented cases. No change to the safety rating was necessary. Clinical reports of SAEs were not available. Pat.-ID 612: Rating of SAE severity was changed from moderate to life threatening due to acute suicidal tendency. Inclusion of patient was questioned because of exclusion criteria.

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DSMB 2 (24.05.2016)	63	14	No influence of the intervention was seen in the presented cases. No change to the safety rating was necessary. Clinical reports of SAEs happening after discharge from inpatient clinic were not available. Pat.-ID 710: Date of SAE incorrect. Pat-ID 211: Date of SAE incorrect.
DSMB 3 (18.04.2017)	50	8	No influence of the intervention was seen in the presented cases. No change to the safety rating was necessary.
DSMB 4 (14.11.2017)	40	31 (incl. findings from reviewed cases)	No influence of the intervention was seen in the presented cases. No change to the safety rating was necessary. Pat.ID 605 (SUE Date 06 Mar 2014): This case is not reported as a SUE, since the reported medical finding „cardiac decompensation" is not considered a SUE as defined in the Phys-Stroke study protocol. Nevertheless, this event will be documented in the Summary Report, because a connection to the intervention might be plausible. Pat. ID 632 (SUE Date 06 May 2015): Case does not have to be rated as a separate SUE, since the transfer to another hospital occurred from a hospital and not from a rehabilitation clinic. Pat. ID 632 (SUE Date 08 May 2015): Case does not have to be rated as a separate SUE, since the transfer to another hospital occurred from an acute clinic and not from a rehabilitation clinic. Pat. ID 653 (SUE Date 22 Feb 2016):

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|	Case does not have to be rated as a SUE, since the patient was brought to the emergency unit after falling and not admitted to the hospital for inpatient care. Pat. ID 719 (SUE Date 02 May 2016): Case does not have to be rated as a separate SUE, since the medical finding of pericardial effusion was incidental during hospitalization and had no further consequences in respect to SUE criteria. Pat. ID 724 (SUE Date 24 June 2016): Case is not a SUE, since the hypertensive decompensation did not result in a transferal and does not have another reason for reporting. Pat. ID 820 (SUE Date 18.09.2017): Case does not have to be rated as a separate SUE, since the transfer to another hospital occurred from a hospital and not from a rehabilitation clinic.

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Recruitment and participant Status

Figure 2: Course of recruitment

Course of recruitment

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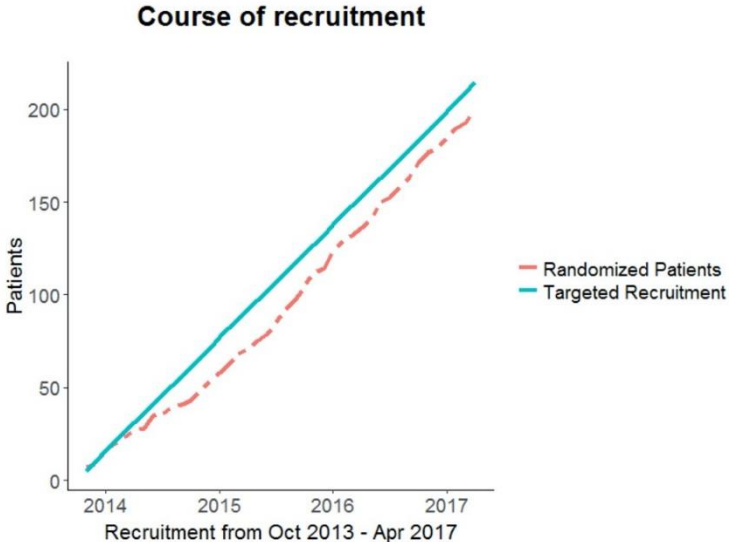
Data Safety Monitoring Board – Summary Report
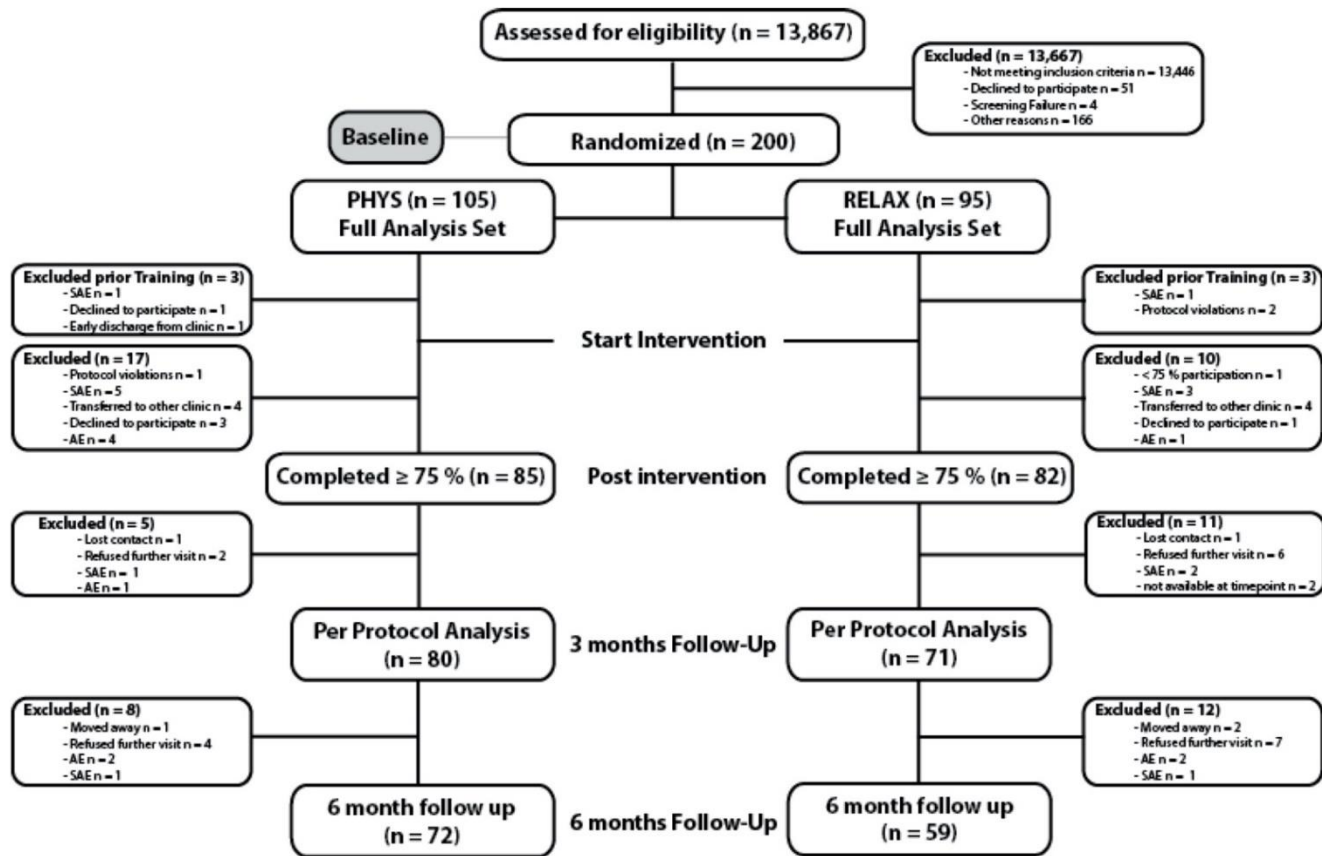- For internal use only -

Figure 3: Recruitment per center
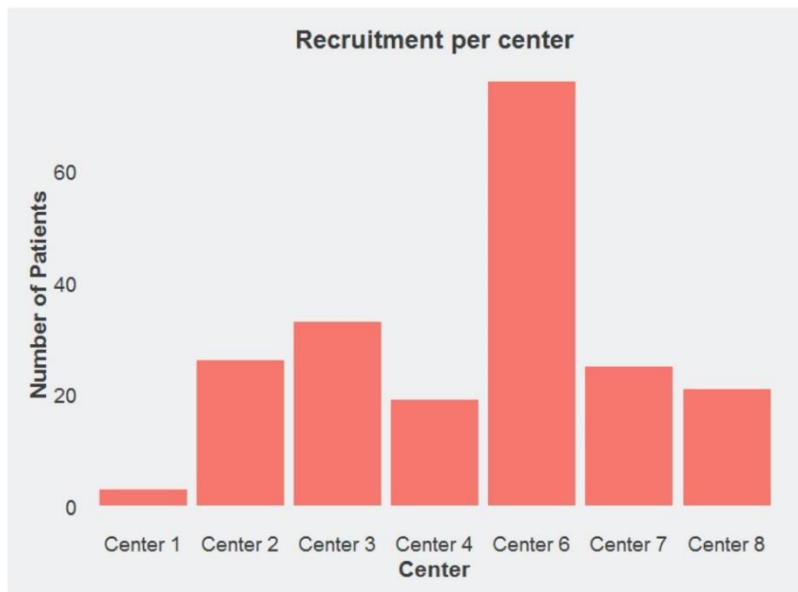
Center 1	Brandenburgklinik Berlin-Brandenburg
Center 2	Evangelisches Geriatriezentrum Berlin
Center 3	Kliniken Beelitz GmbH
Center 4	Median Klinik Grünheide
Center 5	Median Klinik Berlin-Kladow
Center 6	Medical Park Humboldtmühle Berlin
Center 7	Charité Universitätsmedizin Berlin, Campus Benjamin Franklin
Center 8	Vivantes Neukölln

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Figure 4: Flow chart

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Table 3: Screening Failures

Pat-ID	Reason	Randomized
103	Inclusion 124 days after index (protocol deviation)	Yes
104	Screening: Patient not able to comprehend the meaning and purpose of study	No
203	Previously undiagnosed stenosis of esophagus: Taken out of trial by study doctor before randomization	No
206	Fall with fracture one day after Baseline visit prior to intervention phase	Yes
310	Baseline Visit discontinued due to nausea; no randomization due to nausea (see Monitoring Report Beelitz 01.06.2015)	No
325	Patient is discharged from the rehabilitation clinic before intervention can be finished.	Yes
625	Myocardial infarcation < 120 days prior to inclusion (protocol deviation)	No
675	Atrial fibrillation: first diagnosis before 1st day of intervention	Yes
707	Global Aphasia (protocol deviation): noticed at Baseline by Assessor	Yes
724	Patient rejects intervention	Yes

N = 10

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Table 4: Protocol deviations

Pat-ID	Reason	Deviation
103	Inclusion 124 days after index	Major
327	Inclusion 48 days after index	Minor
625	Myocardial infarction < 120 days prior to inclusion	Major
630	Inclusion 48 days after index	Minor
656	Inclusion 46 days after index	Minor
667	Inclusion 47 days after index	Minor
675	Atrial fibrillation: first diagnosis before 1st day of intervention	Major
707	Global Aphasia: noticed at Baseline by Assessor	Major
708	Inclusion 4 days after index	Minor
709	Inclusion 3 days after index	Minor

Patients will still be included if minor violations of inclusion criteria are found such as:

- no imaging is available confirming the stroke but obvious signs of stroke are present

- subjects included earlier than 5 days post stroke, if the intervention did not start before the fifth day post stroke

- subjects included after 45 days post stroke, if the intervention started within 55 days post stroke
(for more information see Statistical Analysis Plan)

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Table 5: Summary of missed visits

Missed visits per center										Total
	Center 1	Center 2	Center 3	Center 4	Center 5	Center 6	Center 7	Center 8		
Baseline	4	26	33	19	0	76	25	21		204
V1	2	8	7	1	0	8	6	1		33
V2	2	9	6	0	0	10	7	2		36
V3	3	12	8	2	0	19	11	4		59

Included patients per center and number of missed visits (V1 – V3). Patients that withdrew from treatment or were lost to follow-up regarding the V2 visit were asked to be still assessed on any upcoming visit.

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Table 6: List Dropouts per site
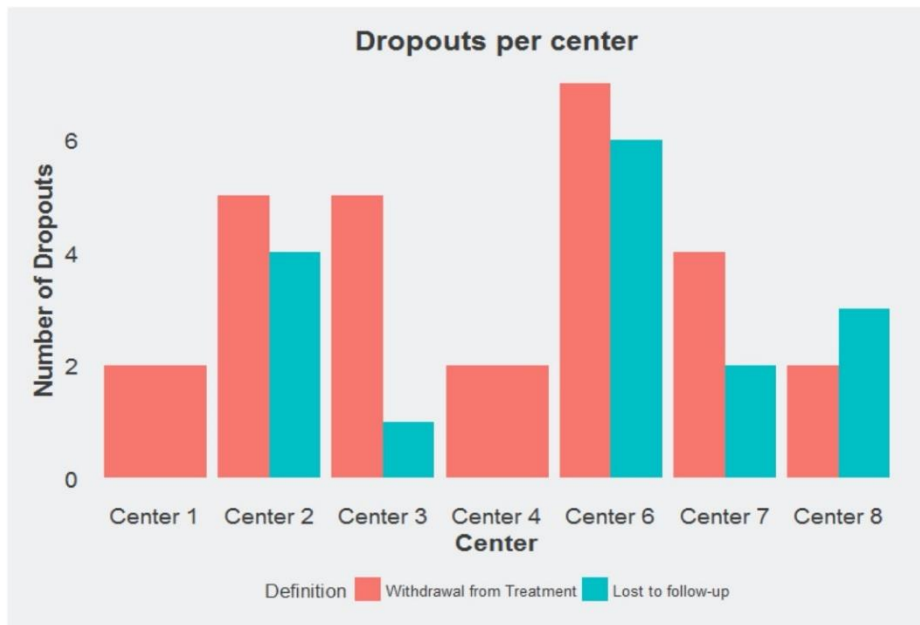
Center 1	Brandenburgklinik Berlin-Brandenburg
Center 2	Evangelisches Geriatriezentrum Berlin
Center 3	Kliniken Beelitz GmbH
Center 4	Median Klinik Grünheide
Center 5	Median Klinik Berlin-Kladow
Center 6	Medical Park Humboldtmühle Berlin
Center 7	Charité Universitätsmedizin Berlin, Campus Benjamin Franklin
Center 8	Vivantes Neukölln

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Participants

Table 6: Baseline Characteristics

Variable	PHYS-Group (n=105)	RELAX-Group (n=95)	All Patients (n=200)
Demographics			
Age in years, mean (SD) [min – max]	69 (12) [21 – 90]	70 (11) [40 – 89]	69 (12) [21 – 90]
Female sex – n. (%)	45 (42.9)	36 (37.9)	81 (40.5)
Education in years, mean (SD) (4 missings)	14 (4)	14 (4)	14 (4)
Occupation			
Retired, n (%)	73 (69.5)	74 (77.9)	147 (73.5)
Employed / self employed / worker, n (%)	24 (22.9)	16 (16.8)	40 (20)
Other (pupil / student, unemployed, housewife / househusband), n (%)	8 (7.6)	5 (5.3)	13 (6.5)

Demographic baseline values of both groups. Values in mean (SD, standard deviation), median and interquartile range (IQR)

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Training Response

Table 7: Training Response

Full analysis Set	PHYS	RELAX
	n = 105	N = 95
Days to start intervention	29 (14)	29 (17)
Intervention duration in days	17 (6)	18 (4)
Time in intervention (min)	21 (4)	24 (3)
Heart Rate pre session (bpm)	78 (10)	73 (10)
Heart Rate post session (bpm)	94 (14)	71 (10)
Blood pressure pre session (mmHg)	126/75 (11/9)	126/74 (12/8)
Blood pressure post session (mmHg)	128/76 (9/7)	123/73 (12/8)
Visual analog scale of perceived exertion	5 (0)	2 (0)
Reason for stopping intervention sessions		
Pain	0 (1)	0 (0)
Urge to urinate	0 (0)	0 (0)
Time	0 (0)	0 (0)
Fatigue	2 (4)	0 (0)
Refusal	0 (0)	0 (0)
Other	0 (1)	0 (0)

Training response values of both groups. Values in mean (SD, standard deviation)

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Safety Assessments

Table 8: Medical conditions of participants at Baseline

Variable	PHYS-Group (n=105)	RELAX-Group (n=95)	All Patients (n=200)
Stroke characteristics, acute therapy			
Ischemic Stroke, n. (%)	91 (86.7)	90 (94.7)	181 (90.5)
Treatment with alteplase, n (only in ischemic stroke patients)	34 (32.7)	27 (28.4)	61 (30.7)
Time from stroke to intervention in days, median (IQR)	30 (17 – 39)	27 (17 – 41)	28 (17 – 40)
Anterior circulation stroke, n (%)	21 (20)	23 (24.2)	44 (22)
NIHSS score, median (IQR) (1 missing)	9 (5 – 12) [1 – 23]	8 (5 – 11) [0 – 20]	9 (5 – 12) [0 – 23]
TOAST (only ischemic patients)			
LAA, n (%)	17 (18.7)	19 (21.1)	36 (19.9)
Cardioembolism, n (%)	18 (19.8)	18 (20)	36 (19.9)
Small vessel occlusion, n (%)	16 (17.6)	15 (16.7)	31 (17.1)
Other etiology, n (%)	3 (3.3)	4 (4.4)	7 (3.9)
Undetermined etiology, n (%)	34 (37.4)	28 (31.1)	62 (34.3)
Competing etiologies, n (%)	3 (3.3)	6 (6.7)	9 (5)

Clinical baseline values of both groups. Values in mean (SD, standard deviation), median and interquartile range (IQR)

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Table 9: Safety parameters

	All N=200	Phys N=105	Relax N=95	IRR (Incidence-Rate-Ratio) (95%CI)	p
Follow up time (in days), median (IQR)	153 (138-165)	154 (139-166)	152 (133-164)		
Stroke IR 95%CI	14 1.54 (0.91-2.59)	10 2.06 (1.11-3.82)	4 0.94 (0.35-2.50)	2.19 (0.69-6.98)	0.186
Myocardial infarction	--	--	--		
Admission to hospital IR 95%CI	26 2.85 (1.94-4.19)	19 3.91 (2.49-6.13)	7 1.64 (0.78-3.45)	2.38 (1.00-5.65)	0.050
Death IR 95%CI	5 0.55 (0.23-1.32)	1 0.21 (0.03-1.46)	4 0.94 (0.35-2.50)	0.21 (0.03-1.46)	0.174

Severe adverse events (number of events) from screening to v3 (or until last observation, if drop out) by group, incidence rates (IR) per 100 patient-months and 95% CI, incidence rate ratio (IRR) and 95%CI for group comparison, based on poisson regression

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| Event	All N = 200	Recurrent stroke N = 14	Referral to acute hospital N = 26	Death N = 5
TOAST (N, % in categories)				
Large-artery atherosclerosis	34 (21.4%)	3 (21 %)	7 (29 %)	2 (40 %)
Cardioembolism	34 (21.4%)	1 (7 %)	12 (50 %)	1 (20 %)
Small-artery occlusion	29 (18.2%)	3 (21 %)	1 (4 %)	1 (20 %)
Other	7 (4.4%)	0 (0 %)	0 (0 %)	0 (0 %)
Unknow aetiology	46 (28.9%)	5 (36 %)	5 (21 %)	0 (0 %)
Competing aetiology	9 (5.7%)	3 (21%)	1 (4 %)	1 (20 %)
Circulation (n, % in categories)				
Anterior circulation	150 (77.3%)	4 (29 %)	7 (29 %)	4 (80 %)
Posterior circulation	47 (24.2%)	11 (79 %)	23 (96 %)	1 (20 %)
Hemisphere (right, left)	105 / 87	10 / 5	19 / 6	3 / 2
NIHSS, Mean (SD)	9 (5)	6 (4)	9 (5)	7 (5)

Medical conditions of patients with SAEs at Baseline grouped by SAE type. Values are in counts (%) or mean (sd). Patients with several SAEs of the same kind were only counted once.

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Figure 5: SAE - Referral to acute hospital

1	Neurology
2	Cardiology
3	Orthopaedics / Traumatology
4	Internal Medicine
5	Tumor center
6	Psychiatry
7	Surgery
8	Other

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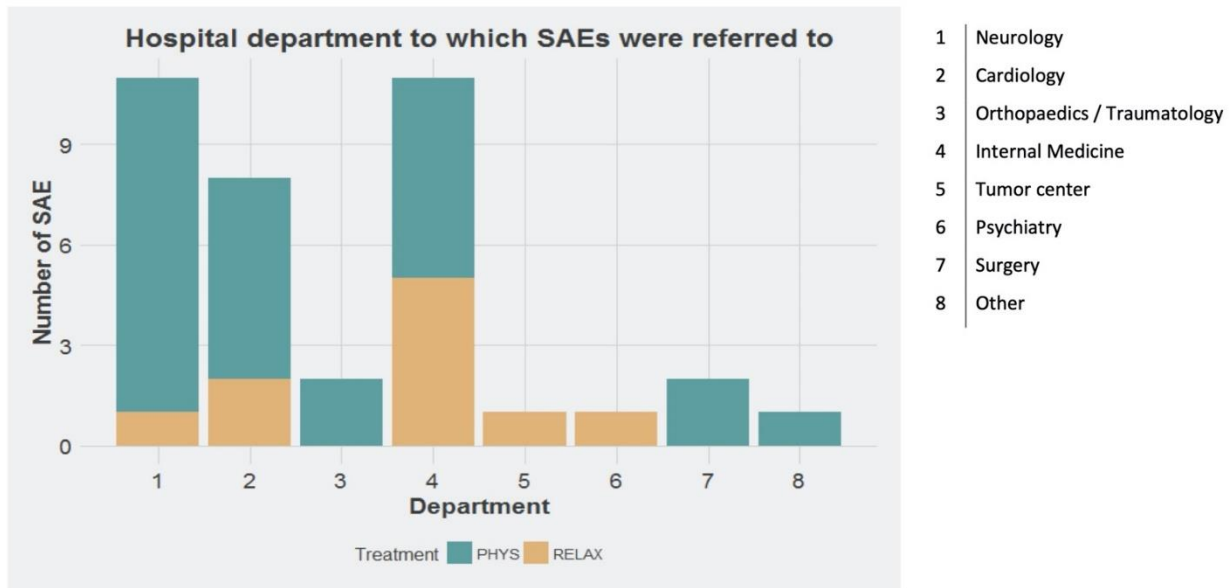
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<u>Figure 6: SAE and AE per center</u>
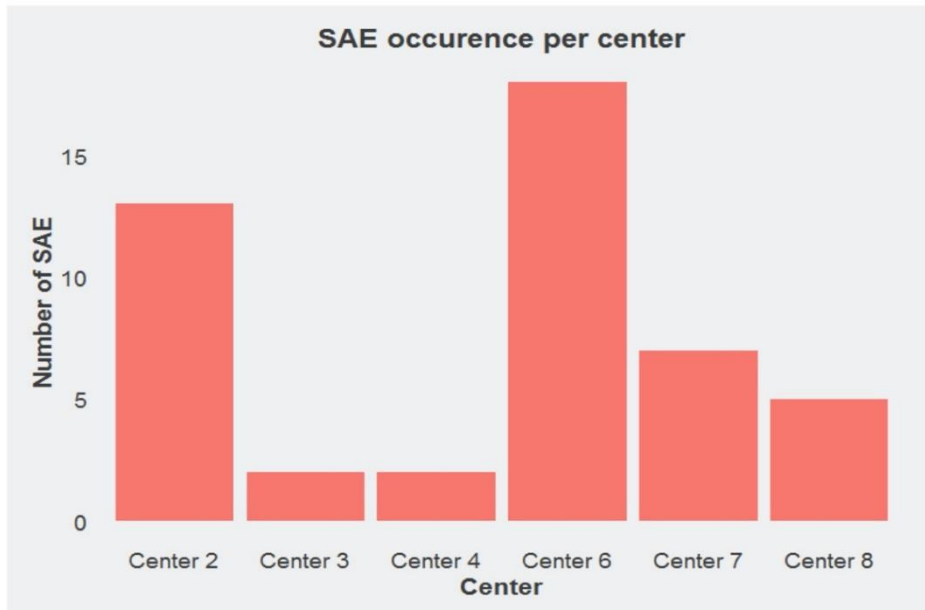
Center 1	Brandenburgklinik Berlin-Brandenburg
Center 2	Evangelisches Geriatriezentrum Berlin
Center 3	Kliniken Beelitz GmbH
Center 4	Median Klinik Grünheide
Center 5	Median Klinik Berlin-Kladow
Center 6	Medical Park Humboldtmühle Berlin
Center 7	Charité Universitätsmedizin Berlin, Campus Benjamin Franklin
Center 8	Vivantes Neukölln

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Figure 7: Timing of SAE

1	Prior to Baseline
2	At Baseline
3	Between Baseline and Intervention
4	During Intervention phase
5	After Intervention before leaving of inpatient rehabilitation
6	After leaving Rehabilitation
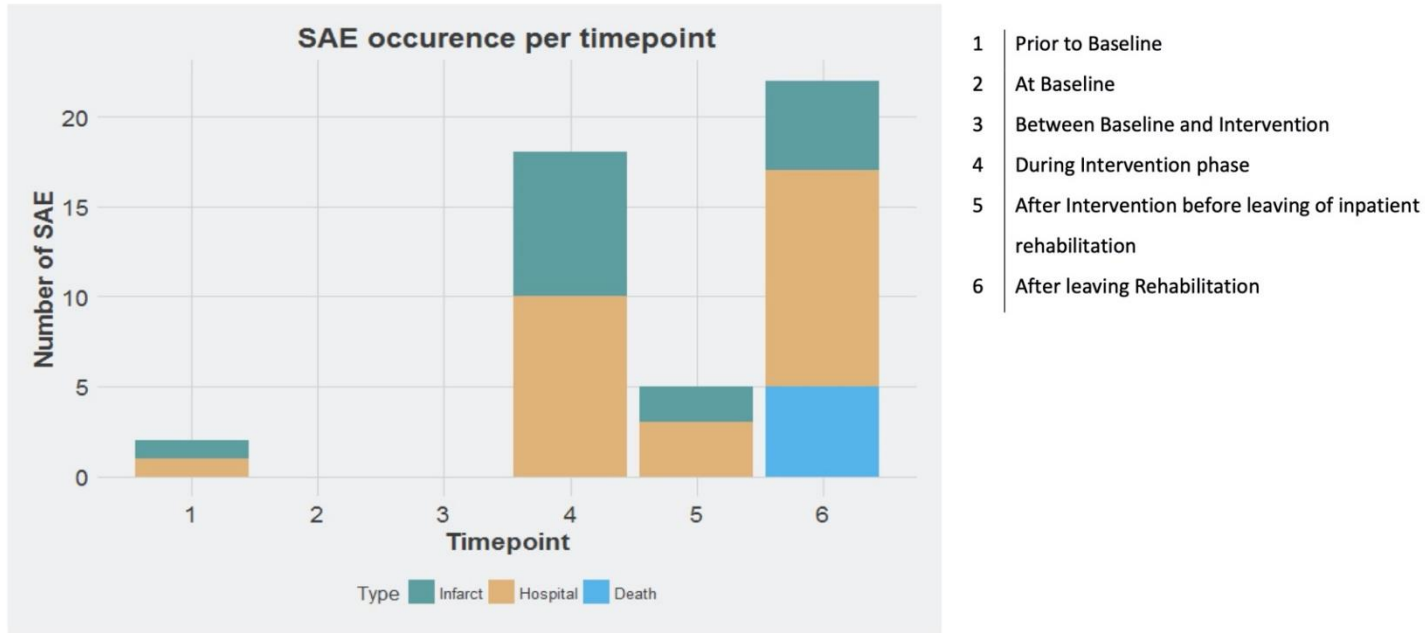
Table 10: Deaths

Participant ID	Date of Death	Cause of Death	Relationship to Intervention
217	24.08.2016	Acute Aorta dissection Stanford Typ A	Unlikely
222	28.04.2017	Urosepsis caused by Klebsiella penumoniae	Unlikely
640	16.02.2016	Unknown	Possible
643	15.03.2016	Ischemic Stroke in thalamus area, cerebellum and bilateral posterior infarct ; septic thromboembolism generally from bacterial endocarditis; sepsis with staphylococcus aureus in blood cultures	Unlikely
722	16.09.2016	Reanimation after re-infarct	Unlikely

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Table 11: Adverse events

	All N=200	Phys N=105	Relax N=95	IRR (Incidence-Rate-Ratio) (95%CI)	p
Follow up (in days), median (IQR)	33 (29-36)	33 (29-36)	33 (28-36)		
Pain IR 95%CI	112 52.76 (43.84-63.49)	68 61.20 (48.25-77.62)	44 43.49 (32.36-58.44)	1.41 (0.96-2.06)	0.077
Fatigue IR 95%CI	50 23.55 (17.85-31.07)	29 26.10 (18.14-37.56)	21 20.76 (13.53-31.83)	1.26 (0.72-2.20)	0.424
Dizziness IR 95%CI	19 8.95 (5.71-14.03)	5 4.50 (1.87-10.81)	14 13.84 (8.19-23.36)	0.33 (0.12-0.90)	0.031
Falls IR 95%CI	50 23.55 (17.85-31.07)	36 32.40 (23.37-44.92)	14 13.84 (8.19-23.36)	2.34 (1.26-4.34)	0.007
Fractures IR per 100 patient-months, 95%CI	--	--	--	--	
Other IR per 100 patient-months, 95%CI	12 5.65 (3.21-9.95)	8 7.20 (3.60-14.40)	4 3.95 (1.48-10.53)	1.82 (0.55-6.05)	0.328

Adverse events from screening to v1 (or until last observation, if drop out) by group, incidence rates

(IR) per 100 patient-months and 95% CI, incidence rate ratio (IRR) and 95%CI for group comparison,

based on poisson regression

Definitions

Pain, fatigue and dizziness were considered an adverse events if it was described as a strong and severe sensation and lasted or occurred several times over a period of 24 hrs.

Consequences of falls

Consequences were only reported in 5 cases (2x no injury, 1x abrasion, 1x pain, 1x tension) in the RELAX group. The PHYS group reported consequences in 7 cases (3x abrasion, 3x pain, 1x hematoma).

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8. Eidesstattliche Versicherung

„Ich, Torsten Rackoll, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „ Efficacy and harms of a four week aerobic gait training compared to relaxation therapy in patients with subacute stroke" bzw. „Wirksamkeit und Nebenwirkungen eines vierwöchigen aeroben Gangtrainings im Vergleich zu einer Entspannungstherapie bei Patienten nach subakutem Schlaganfall" (deutschsprachiger Titel) selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.
Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren/innen beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) werden von mir verantwortet.

Ich versichere ferner, dass ich die in Zusammenarbeit mit anderen Personen generierten Daten, Datenauswertungen und Schlussfolgerungen korrekt gekennzeichnet und meinen eigenen Beitrag sowie die Beiträge anderer Personen korrekt kenntlich gemacht habe (siehe Anteilserklärung). Texte oder Textteile, die gemeinsam mit anderen erstellt oder verwendet wurden, habe ich korrekt kenntlich gemacht.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Erstbetreuer/in, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; www.icmje.og) zur Autorenschaft eingehalten. Ich erkläre ferner, dass ich mich zur Einhaltung der Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis verpflichte.

Weiterhin versichere ich, dass ich diese Dissertation weder in gleicher noch in ähnlicher Form bereits an einer anderen Fakultät eingereicht habe.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§§156, 161 des Strafgesetzbuches) sind mir bekannt und bewusst."

Datum   Unterschrift
01.12.2021

9. Anteilserklärung an den erfolgten Publikationen

Torsten Rackoll hatte folgenden Anteil an den folgenden Publikationen:

Publikation 1:
Nave AH, **Rackoll T**, Grittner U, Bläsing H, Gorsler A, Nabavi DG, Audebert HJ, Klostermann F, Müller-Werdan U, Steinhagen-Thiessen E, Meisel A, Endres M, Hesse S, Ebinger M, Flöel A. *Physical Fitness Training in Patients with Subacute Stroke (PHYS-STROKE): multicentre, randomised controlled, endpoint blinded trial.* BMJ. 2019;366:l5101. doi:10.1136/bmj.l5101

<u>Beitrag im Einzelnen:</u>
Bei der von Herrn Rackoll und Herrn Dr. Nave in geteilter Erstautorenschaft erstellten Publikation handelt es sich um eine multizentrische, randomisierte kontrollierte Studie, die vom Centrum für Schlaganfallforschung Berlin durchgeführt und von der Substudie BAPTISe begleitet wurde. Die Studienleitung der PHYS-STROKE Studie oblag Frau Prof. Dr. Flöel und die Studienleitung der begleitenden BAPTISe Studie Herrn Prof. Dr. Ebinger. Herr Rackoll beteiligte sich an der Planung der Studie und übernahm die Studienkoordination der PHYS-STROKE Studie, während Herr Nave die Studienkoordination der BAPTISe Studie innehatte. Zu den Aufgaben von Herrn Rackoll gehörten neben dem Studienmanagement, die Einarbeitung der Studienassessoren in der Spirometrie, die Qualitätssicherung der Erhebungen sowie in Zusammenarbeit mit der Biostatistikerin Frau PD. Dr. Grittner die Erstellung des statistischen Analyseplans (Anteilig erstellt: Torsten Rackoll (45%), Ulrike Grittner (45%), Alexander Nave (10%)), sowie die deskriptive Statistik und Erstellung der Grafiken.

Folgend sind die anteilig erbrachten Leistungen für die Bereiche des vorliegenden Manuskripts dargestellt:

Text:	Alexander Nave (90%), Torsten Rackoll (10%)
Statistik:	Ulrike Grittner (Treatment effects), Torsten Rackoll (deskriptive Statistik)
Supplemental appendix:	Torsten Rackoll
Table 1:	Torsten Rackoll (Darstellung und deskriptive Statistik)
Table 2:	Ulrike Grittner
Table 3:	Torsten Rackoll (Deskriptive Statistik), Ulrike Grittner (Treatment effects)
Table 4:	Ulrike Grittner
Figure 1:	Torsten Rackoll
Figure 2:	Torsten Rackoll
Figure 3:	Torsten Rackoll
Figure 4:	Torsten Rackoll (Darstellung), Ulrike Grittner (Statistik)
Figure 5:	Ulrike Grittner

Publikation 2:

Rackoll T, Nave AH, Ebinger M, Endres M, Grittner U, Flöel A; for the PHYS-Stroke study group. *Physical Fitness Training in Patients with Subacute Stroke (PHYS-STROKE): Safety analyses of a randomized clinical trial.* Int J Stroke. 2021 Apr 7:17474930211006286.

doi: 10.1177/17474930211006286.

Beitrag im Einzelnen:

Die Überwachung der schwerwiegenden, unerwarteten Ereignisse während des Studienverlaufs sowie die Berichterstattung gegenüber dem Data Safety Monitoring Board wurde durch Herrn Rackoll durchgeführt. Im Vorfeld zur Erstellung des Manuskripts wurde die Analyse geplant (Anteilig durch: Torsten Rackoll (60%) und Alexander Nave (40%)).

Folgend sind die anteilig erbrachten Leistungen für die Bereiche des vorliegenden Manuskripts dargestellt:

Text:	Torsten Rackoll (80%) und Alexander Nave (20%)
Statistik:	Torsten Rackoll
Table 1:	Torsten Rackoll
Figure 1:	Torsten Rackoll
Figure 2:	Torsten Rackoll
Figure 3:	Torsten Rackoll

Manteltext:

Die unveröffentlichten MRT-Analysen sowie die Therapeut:inneninterviews wurden von Torsten Rackoll geplant und unterteilen sich folgendermaßen:

Konzeptionierung:	Torsten Rackoll
Therapeut:inneninterviews	Torsten Rackoll
Statistik:	Torsten Rackoll
Läsionsmaskierung:	Hamza Moussa, Dr. Kersten Villringer, Torsten Rackoll
Datenanalyse	Andrea dell Orco, Torsten Rackoll
Table 4:	Torsten Rackoll
Figure 2:	Torsten Rackoll

_____

Unterschrift des Doktoranden

10. Publikation 1: Physical Fitness Training in Patients with Subacute Stroke (PHYS-STROKE): multicentre, randomised controlled, endpoint blinded trial

Nave AH, **Rackoll T**, Grittner U, Bläsing H, Gorsler A, Nabavi DG, Audebert HJ, Klostermann F, Müller-Werdan U, Steinhagen-Thiessen E, Meisel A, Endres M, Hesse S, Ebinger M, Flöel A. *Physical Fitness Training in Patients with Subacute Stroke (PHYS-STROKE): multicentre, randomised controlled, endpoint blinded trial.* BMJ. 2019;366:l5101. doi:10.1136/bmj.l5101

Shared first-authorship: Nave & Rackoll

Journal Data Filtered By: **Selected JCR Year: 2017** Selected Editions: SCIE,SSCI
Selected Categories: **"MEDICINE, GENERAL and INTERNAL"**
Selected Category Scheme: WoS
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1	NEW ENGLAND JOURNAL OF MEDICINE	332,830	79.258	0.702000
2	LANCET	233,269	53.254	0.435740
3	JAMA-JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION	148,774	47.661	0.299960
4	BMJ-British Medical Journal	109,303	23.259	0.150320
5	JAMA Internal Medicine	11,840	19.989	0.076280
6	ANNALS OF INTERNAL MEDICINE	53,689	19.384	0.099140

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Physical Fitness Training in Patients with Subacute Stroke (PHYS-STROKE): multicentre, randomised controlled, endpoint blinded trial

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ABSTRACT

OBJECTIVE
To determine the safety and efficacy of aerobic exercise on activities of daily living in the subacute phase after stroke.

DESIGN
Multicentre, randomised controlled, endpoint blinded trial.

SETTING
Seven inpatient rehabilitation sites in Germany (2013-17).

PARTICIPANTS
200 adults with subacute stroke (days 5-45 after stroke) with a median National Institutes of Health stroke scale (NIHSS, range 0-42 points, higher values indicating more severe strokes) score of 8 (interquartile range 5-12) were randomly assigned (1:1) to aerobic physical fitness training (n=105) or relaxation sessions (n=95, control group) in addition to standard care.

INTERVENTION
Participants received either aerobic, bodyweight supported, treadmill based physical fitness training or relaxation sessions, each for 25 minutes, five times weekly for four weeks, in addition to standard rehabilitation therapy. Investigators and endpoint assessors were masked to treatment assignment.

MAIN OUTCOME MEASURES
The primary outcomes were change in maximal walking speed (m/s) in the 10 m walking test and change in Barthel index scores (range 0-100 points, higher scores indicating less disability) three months after stroke compared with baseline. Safety outcomes were recurrent cardiovascular events, including stroke, hospital readmissions, and death within three months after stroke. Efficacy was tested with analysis of covariance for each primary outcome in the full analysis set. Multiple imputation was used to account for missing values.

RESULTS
Compared with relaxation, aerobic physical fitness training did not result in a significantly higher mean change in maximal walking speed (adjusted treatment effect 0.1 m/s (95% confidence interval 0.0 to 0.2 m/s), P=0.23) or mean change in Barthel index score (0 (−5 to 5), P=0.99) at three months after stroke. A higher rate of serious adverse events was observed in the aerobic group compared with relaxation group (incidence rate ratio 1.81, 95% confidence interval 0.97 to 3.36).

CONCLUSIONS
Among moderately to severely affected adults with subacute stroke, aerobic bodyweight supported, treadmill based physical fitness training was not superior to relaxation sessions for maximal walking speed and Barthel index score but did suggest higher rates of adverse events. These results do not appear to support the use of aerobic bodyweight supported fitness training in people with subacute stroke to improve activities of daily living or maximal walking speed and should be considered in future guidelines.

TRIAL REGISTRATION
ClinicalTrials.gov NCT01953549.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Current guidelines endorse cardiorespiratory training within post-stroke rehabilitation programmes

Large randomised controlled trials of this recommendation are scarce, resulting in inconclusive data on efficacy for disability (activities of daily living) and safety of physical fitness training after stroke

WHAT THIS STUDY ADDS

Results suggest that in adults with moderate to severe subacute stroke, in addition to standard rehabilitation care, aerobic, bodyweight supported, treadmill based fitness training did not improve maximal walking speed or activities of daily living compared with relaxation

The rate of serious adverse events was higher in the aerobic physical fitness training group than relaxation group

Compared with current guideline recommendations, these results do not appear to support the use of aerobic, bodyweight supported, treadmill based fitness training in this stroke population

Introduction

Despite encouraging advances in the early treatment of stroke,[1] at least one third of the 10 million people worldwide with new stroke each year[2] remain functionally dependent and as a result experience impairments in activities of daily living.[3 4] The number of stroke survivors with impairments in activities of daily living is increasing, leading to more people with stroke who are dependent on rehabilitation interventions.[5] To date, no drug treatments are available to enhance rehabilitation. Treadmill based physical fitness training constitutes a non-drug approach in stroke rehabilitation that might not only prevent deconditioning but also show associated

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RESEARCH

benefits on activities of daily living, such as walking and climbing stairs.[6-8]

A meta-analysis of small randomised controlled trials showed improvements in speed and tolerance of walking after physical fitness training in stroke survivors.[6] The studies have, however, varied in type and intensity of exercise, timing of initiation after stroke, and control groups.[7 9-11] The American Heart Association/American Stroke Association currently recommends aerobic exercise for stroke survivors, with three to five sessions weekly lasting 20 to 60 minutes and at a heart rate of 55-80% of the maximum. Applied in the subacute stage after stroke, aerobic exercise is thought to promote neuroplasticity and to have beneficial effects on functional outcomes.[7] So far, nine randomised controlled trials (n=324) have compared the effects of aerobic physical fitness training on maximal walking speed—an important indicator of mobility in everyday life[6]—with a control intervention. However, only two of these studies (n=73) enrolled participants within the first six weeks after stroke, and these participants showed improvement in maximal walking speed post-intervention.[6] For activities of daily living and disability, two small randomised rehabilitation trials (n=199) that applied 400-540 minutes of physical fitness training in the early and late subacute stage after stroke found an increase in the Barthel index score, a disability scale widely used in the clinical setting to measure activities of daily living.[12 13] Meta-analyses have, however, indicated that the evidence for improvement in Barthel index scores after physical fitness training is still inconclusive.[6]

Previously, the larger Locomotor Experience Applied Post-Stroke (LEAPS) trial randomised 408 participants to treadmill based locomotor training either two or six months after stroke, or to a progressive exercise programme at home, and did not detect a difference in treatment effects.[11] LEAPS did not, however, apply an aerobic physical fitness training early after stroke.

We performed a multicentre, randomised controlled trial in adults with stroke in the early subacute phase (days 5-45 after stroke) to determine the efficacy of aerobic treadmill based, physical fitness training on maximal walking speed and activities of daily living compared with relaxation as a control intervention.
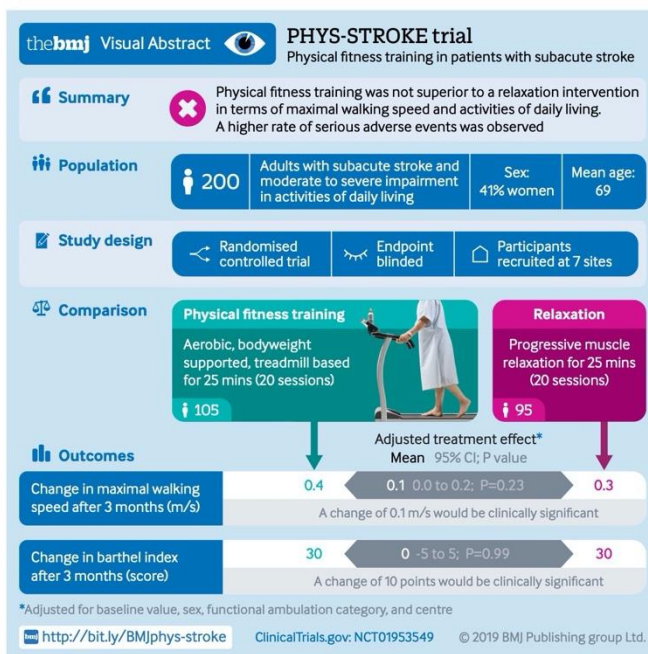
Methods

Study design

The study protocol for the multicentre, randomised controlled, endpoint blinded Physical Fitness Training in Patients with Subacute Stroke (PHYS-STROKE) trial is available online (https://doi.org/10.1186/1745-6215-15-45) and has been published previously.[14] All participants provided informed consent. Adults were enrolled at seven inpatient rehabilitation sites in Berlin, Germany, and the surrounding area. A medical monitoring and independent data safety and monitoring board was appointed by the Centre for Stroke Research Berlin. Guidance of study centre representatives was maintained by regular telephone contact and study visits by members of the coordinating trial centre at the Centre for Stroke Research Berlin. To determine eligibility for the trial, trained trial physicians screened people with an imaging confirmed diagnosis of ischaemic or haemorrhagic stroke who had been admitted to hospital within a recruiting centre.

Participants

People were eligible for the trial if they were aged at least 18 years, were in the subacute phase of ischaemic or haemorrhagic stroke (days 5-45 after stroke onset), were able to sit unsupported for at least 30 seconds, were considered able to perform aerobic exercise by the responsible trial physician, and had a Barthel index score of 65 or less at the time of enrolment. The Barthel index measures activities of daily living based on 10 items, with scores ranging from 0 to 100 points—higher scores indicating less dependence.[15] Key exclusion criteria were intracranial haemorrhage from a ruptured aneurysm or arteriovenous malformation, inability to perform required physical exercise, assisted walking before stroke, or severe cardiac or psychiatric comorbidities. If study requirements were met, trial physicians assessed information on stroke type and medical conditions after written informed consent was obtained. The supplementary file lists the inclusion and exclusion criteria.

Randomisation and blinding

Participants were stratified by age (≤65 years, >65 years), functional ambulation category (scores ≤3, >3), and centre and were randomised using a web based tool in a 1:1 ratio to receive aerobic physical

the bmj Visual Abstract — **PHYS-STROKE trial**
Physical fitness training in patients with subacute stroke

Summary — Physical fitness training was not superior to a relaxation intervention in terms of maximal walking speed and activities of daily living. A higher rate of serious adverse events was observed

Population — 200 — Adults with subacute stroke and moderate to severe impairment in activities of daily living — Sex: 41% women — Mean age: 69

Study design — Randomised controlled trial — Endpoint blinded — Participants recruited at 7 sites

Comparison
Physical fitness training — Aerobic, bodyweight supported, treadmill based for 25 mins (20 sessions) — 105
Relaxation — Progressive muscle relaxation for 25 mins (20 sessions) — 95

Outcomes
Adjusted treatment effect* — Mean — 95% CI; P value
Change in maximal walking speed after 3 months (m/s) — 0.4 — 0.1 0.0 to 0.2; P=0.23 — 0.3 — A change of 0.1 m/s would be clinically significant
Change in barthel index after 3 months (score) — 30 — 0 -5 to 5; P=0.99 — 30 — A change of 10 points would be clinically significant

*Adjusted for baseline value, sex, functional ambulation category, and centre
http://bit.ly/BMJphys-stroke — ClinicalTrials.gov: NCT01953549 — © 2019 BMJ Publishing group Ltd.

fitness training or relaxation sessions (control group) in addition to standard care.[16] The functional ambulation category is a six point scale that assesses dependency on walking aids, with higher scores indicating less dependency. Trained study assessors (physiotherapists, occupational therapists) collected information on participant characteristics and clinical data from chart reviews at baseline visits and carried out outcome assessments at baseline and follow-up. Study assessors and the trial statistician were blinded to the intervention allocation.

Interventions

Therapists at each rehabilitation site performed interventions according to standardised protocols taught by a manual and two day training course. The study interventions were applied during inpatient stay at the rehabilitation centre, in addition to standard rehabilitation therapy according to German guidelines (www.bar-frankfurt.de). The supplementary file provides detailed information on the amount and content of standard care. Each study session was for 50 minutes (therapist led), comprising 25 minutes of core intervention (training aimed at target heart rate or relaxation time), and took place five times weekly over four weeks (20 sessions in total). The start and end of each intervention session as well as the duration of the core intervention were documented for each participant. An intervention period of four weeks was chosen to ensure intervention sessions were applied during the length of the inpatient stay at the rehabilitation centres. This also enabled the assessment of short term outcomes at three months after stroke.

The supplementary appendix provides a detailed description of the aerobic physical fitness training and relaxation interventions. Briefly, the aerobic physical fitness training sessions included treadmill based, bodyweight supported training at a cardiorespiratory active (aerobic) level to reach a target heart rate for 25 minutes. The target heart rate was calculated by the formula 180 minus years of age, a pragmatic decision, which resembles conventional approaches for calculation of target heart rate that is 50-60% of each participant's maximum heart rate (see supplementary appendix). If participants used β blockers, we reduced the target heart rate by 10 beats per minute. Participants used a bodyweight supported treadmill (Multi-disk treadmill Callis, Model Therapie; Sprintex Trainingsgeräte, Kleines Wiesental, Germany; Reha-Stim, Berlin, Germany) if their functional ambulation category score was 3-5 or an electromechanical gait trainer (Gait Trainer GT1; Reha-Stim, Berlin, Germany) if their score was 0-2. The amount of bodyweight support was applied as required. If necessary, one or two therapists assisted with leg movement, such as extending hip and knee, shifting body weight, or setting the paretic leg in the case of severe paresis of the peroneus muscles. Participants used the same orthoses during the intervention as during standard care physiotherapy. Each training session, including preparation time and a warm-up and cool-down

phase, lasted 50 minutes and comprised 25 minutes of active training at aimed target heart rate, depending on each participant's ability, as recommended in current guidelines.[7] To ensure target heart rate was maintained, heart rate during training was controlled through a pulse sensor (Polar FT1 HRM, Polar Electro Oy, Kempele, Finland) and a screen attached to the treadmill or gait trainer. Reduction of bodyweight support and an increase of belt speed or increase of inclination, or both was used to reach the target heart rate throughout the four week intervention, and thus to constantly induce aerobic training effects. The trainers documented changes of these variables during the intervention period and individual perceived exertion after each training session in intervention diaries. To prevent falls, participants were equipped with a modified parachute harness (Belt system; Reha-Stim, Berlin, Germany).

Relaxation sessions were performed as an active control and focused on contraction and relaxation of muscle groups in the face, arms, shoulders, back, and abdomen for 25 minutes. Participants were instructed to contract the muscles for five to 10 seconds then to relax for 30-40 seconds and were encouraged to pay attention to the feelings of warmth and heaviness. Sessions aimed to promote mental and physical relaxation and avoid any cardiovascular stress. Participants' heart rates were monitored during the relaxation sessions, and ratings of perceived exertion were assessed at the end of each session. Participants in both groups received individual attention during each session to achieve comparability.

No specific treatment policy was prescribed after the intervention period. If participants stopped the intervention prematurely, we continued clinical follow-up at set time points. Participants were analysed in the per protocol analysis if they received 75% or more of the scheduled intervention sessions, had not suspended the intervention for more than five consecutive days, and had participated in the follow-up visit three months after stroke.

Outcome measures

Study visits for outcome assessment were performed before and after the intervention period as well as three and six months after stroke. The primary outcome measures were change in maximal walking speed (assessed in m/s) and change in Barthel index scores three months after stroke compared with baseline in the intention-to-treat analysis. Maximal walking speed was assessed in a 10 m walk test.[17] Participants were asked to walk at maximal speed for 14 m—2 m for acceleration and 2 m for deceleration, with markings on the floor for starting point, at 2 m (start of measurement) and 12 m (end of measurement). The time taken for the walk was measured manually using an electronic time watch for all participants and was additionally controlled with a light beam to trigger an additional watch (Wilhelm Köster Ingenieur für Zeitmessung, Ditzingen, Germany). The test was performed twice and mean speed calculated to avoid

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test-retest error. Prespecified secondary outcomes included the six minute walk test (in metres), Rivermead mobility index[18] (range 0-15, with higher scores indicating better mobility), actigraphy for 24 hours (in steps daily, assessed with GT3x; Actigraph, Pensacola, FL), modified Rankin scale (mRS) score (a scale assessing disability after stroke; ranging from 0 for no symptoms to 6 for death), as well as measures of cognition, motor function, spasticity, mood, and sleep at follow-up compared with baseline. Additionally, in a per protocol dataset we analysed changes in maximal walking speed and Barthel index score three months after stroke compared with baseline. Prespecified biomarker and imaging outcome measures will be analysed separately within an accompanying study,[19] as described in the statistical analysis plan. The supplementary appendix describes the assessments of secondary outcomes. Main prespecified subgroup analyses included dichotomisation of impairment scores (National Institutes of Health stroke scale (NIHSS)[20] assessed on days 3-5 after stroke, a scale that measures neurological deficits, ranging from 0 to 42 points, with higher values indicating more severe strokes, and functional ambulation category), type of stroke, time of study inclusion, and age and sex of participants. We carried out additional post hoc exploratory analyses of differential treatment effects for continuous variables using splines.[21]

Primary safety outcomes included the serious adverse events of recurrent cardiovascular events, including stroke, admission to an acute care hospital, or death within three months after stroke. Trial physicians reported these outcomes to the coordinating trial centre within 24 hours. The adverse events of self reported pain, fatigue, dizziness, and number and nature of falls during the intervention period were recorded after each intervention session. We reported adverse events to the data safety monitoring board on a regular basis.

Statistical analysis

To show the superiority of the aerobic physical fitness training over relaxation intervention, the study was powered on the primary outcome measures to detect a clinically meaningful difference of 0.13 m/s in maximal walking speed (common standard deviation of 0.25 m/s) or 10 points in Barthel index score (common standard deviation of 21 points).[14] Assumptions were based on reported clinical differences from a previous study of another working group (n=155).[13] Overall, 172 participants (86 in each group) were needed to provide 80% power to detect a statistically significant treatment effect for each of the primary endpoints (maximal walking speed and Barthel index score at three months compared with baseline; two sided significance level α=0.025 for each primary outcome, two sample t test). Accounting for a 20% dropout rate, we planned a total sample size of 215 participants. The predefined analyses have been performed as described in the statistical analyses plan of the trial (version 1.0, available online) and were conducted using SPSS, STATA, and R statistical software.[22-24]

All randomised participants were included in the full dataset for the intention-to-treat analysis. Group differences for each primary outcome were analysed using two separate analyses of covariance, with baseline measures as covariates and an additional random effect (random intercept model) to account for clustering of participants in centres. The primary outcomes at follow-up (maximal walking speed and Barthel index score three months after stroke) were the dependent variables in these analyses, and baseline scores and intervention group were independent variables. Additionally, we adjusted the analyses for age, sex, and functional impairment (as assessed by the functional ambulation category test). We imputed missing data because of attrition by using multivariate imputation by chained equations (mice) based on 10 imputed datasets and relevant information generated by the R package mice.[25] The supplementary appendix provides detailed descriptions of data imputation and handling missing data. If we were unable to assess data on mobility measures at baseline because of severe impairment, reasonable single value imputation was carried out by using half the speed of the slowest participant in the group. Analyses of safety endpoints were done using Poisson regression models, which account for the time each participant is at risk and allows incidence rate and incidence rate ratios with confidence intervals to be calculated.

Prespecified subgroup analyses for the primary outcomes were exploratory for sex, age groups, type of stroke, impairment measured by NIHSS and functional ambulation category, and time from stroke to start of intervention. For each subgroup analysis, we tested the interaction between treatment allocation and subgroup to test whether any difference in treatment effect between subgroups was substantial. For each subgroup we also provided mean treatment effects and 95% confidence intervals.

Secondary outcome measures were analysed using a three level mixed model where the repeated measures were nested in participants and participants were nested in centres. We used baseline variables of outcomes as covariates. Models were additionally adjusted for age, sex, and baseline functional ambulation category. In all models we included interaction terms for time point and treatment group. Reported effect estimates were calculated using post-estimation procedures. All models (except for mRS and functional ambulation category) are based on 10 imputations with chained equations and groupwise imputation (see supplementary table S3). For the mRS and functional ambulation category, we used an ordinal logistic mixed regression model including respective covariates for adjustment and random intercept to account for repeated measures. The group variable for centre was entered as fixed covariate in the ordinal mixed models to avoid instability of models. Variables with non-normal distribution were log transformed before analyses. All secondary analyses were carried out in an exploratory framework. No adjustment for multiple testing was applied for secondary analyses.

Patient and public involvement

Before and during the trial, a patient representative appointed by the Berlin Stroke Alliance participated in meetings of the trial's executive steering committee. A member of the trial centre informed the Berlin Stroke Alliance (including participants and family members) about the progress of the trial on a regular basis. After the last trial visit, each participant received a summary of his or her personal outcome measures and blood test results. Findings of the trial will be shared with all participants by providing access to the full manuscript. The supplementary appendix provides a section for participants and carers who provided additional information.

Results

From 26 September 2013 to 30 April 2017, a total of 12 866 adults were screened of whom 200 were included in the trial and underwent randomisation (105 were assigned to aerobic physical fitness training
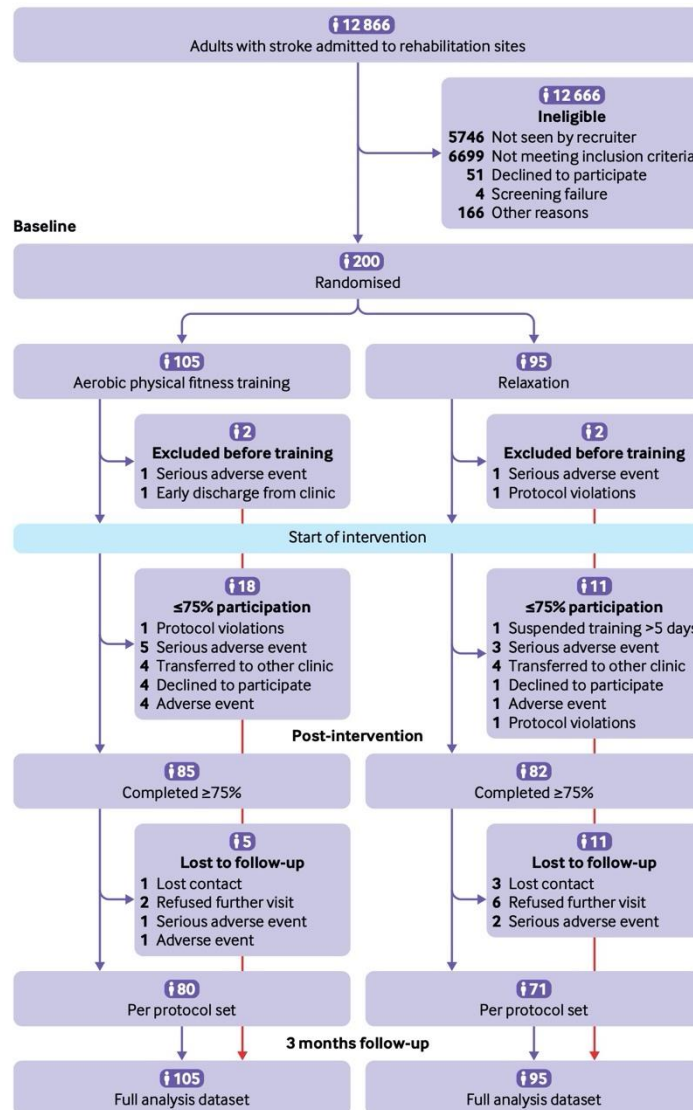
Fig 1 | Flowchart of enrolment and randomisation. Multiple imputation was performed for intention-to-treat analyses of full analysis dataset

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Table 1 | Baseline characteristics of participants stratified by aerobic physical fitness training or relaxation sessions (control group). Values are numbers (percentages) unless stated otherwise

Characteristic	Aerobic physical fitness training (n=105)	Relaxation sessions (n=95)	Total cohort (n=200)
Mean (SD) age (years)	69 (12)	70 (11)	69 (12)
Women	45 (43)	36 (38)	81 (41)
Median (interquartile range) time from stroke to intervention (days)*	30 (17-39)	27 (17-41)	28 (17-40)
Anterior circulation stroke	84 (80)	72 (76)	156 (78)
Hemiparesis on admission	98 (93)	89 (94)	188 (94)
Median (interquartile range) NIHSS score†	9 (5-12)	7 (5-11)	8 (5-12)
Ischaemic stroke	91 (87)	90 (95)	181 (91)
Treatment with alteplase‡	34 (37)	27 (30)	61 (34)
Cause of ischaemic stroke‡:			
Large artery atherosclerosis	17 (19)	19 (21)	36 (20)
Cardioembolism	18 (20)	18 (20)	36 (20)
Small vessel occlusion	16 (18)	15 (17)	31 (17)
Other causes	3 (3)	4 (4)	7 (4)
Undetermined causes	34 (37)	28 (31)	62 (34)
Competing causes	3 (3)	6 (7)	9 (5)
Previous stroke	27 (27)	27 (28)	54 (27)

NIHSS=National Institutes of Health stroke scale.
*Four participants were excluded at screening.
†Scores range from 0 to 42, with higher scores indicating greater stroke severity. Assessed on days 3-5 after stroke. Hospital chart was missing for one participant.
‡Reported proportions of participants treated with alteplase and proportions of causes of stroke refer only to participants with ischaemic stroke.

and 95 to relaxation sessions (control group) in addition to standard care (fig 1). The main reasons for exclusion were a Barthel index score greater than 65, stroke onset more than 45 days before screening, and presence of comorbidities at the time of screening (mainly cardiac). The full analysis set entered the intention-to-treat analysis. The mean age of the study cohort was 69 (SD 12) years and 41% were women. The median NIHSS score was 8 (interquartile range 5-12) points at days 3-5 after stroke. Baseline personal and clinical characteristics were similar in both study groups, except for the six minute walk test and Rivermead mobility index, which were higher in the relaxation group. Table 1 lists the baseline characteristics. The amount of physiotherapy applied during inpatient and outpatient standard care between baseline and follow-up visit three months after stroke was similar between groups (see supplementary table S14).

The median time from stroke onset to start of intervention was 28 (interquartile range 17-40) days. Adherence to the study protocol was good to moderate (fig 2). The mean delta heart rate (pre-post intervention) was 15 (SD 9) beats per minute (bpm) in participants assigned to aerobic physical fitness training compared with −2 (SD 3) bpm in participants assigned to relaxation. Among participants randomised to aerobic physical fitness training, the median percentage of training sessions performed at target heart rate was 70% (interquartile range 23-100%) with improved adherence towards the end of the intervention period (fig 2). The amount of therapy applied during standard care was similar for severely impaired participants (functional ambulation category 0-1) compared with less severely impaired participants (>1), indicating similar treatment

conditions (see supplementary table S7). Participants in the aerobic physical fitness training group received a mean of 16 (SD 6) sessions with a mean duration of the core intervention of 21 (SD 4) minutes, compared with a mean 17 (SD 5) sessions and mean duration of 24 (SD 3) minutes in the relaxation group. Eighteen participants in the aerobic physical fitness training group (17%) did not reach the required amount of 15 intervention sessions, compared with 11 participants (12%) in the relaxation group. Main reasons for termination of the intervention were early transfer to a non-participating hospital (n=4, each group) and a serious adverse event (n=5 in aerobic physical fitness training group; n=3 in relaxation group, see fig 1). At three months after stroke, 34 participants (17%) were lost to follow-up, and respective data were imputed for the intention-to-treat analysis using multiple imputation methods (see supplementary appendix for detailed description). The prespecified per protocol analysis comprised data for 151 participants (76% of the full analysis set), 80 participants in the aerobic physical fitness training group and 71 in the relaxation group.

Table 2 lists the primary efficacy outcomes. The adjusted mean change of maximal walking speed from baseline to three months after stroke was 0.4 m/s (95% confidence interval 0.3 to 0.4 m/s) in the aerobic physical fitness training group and 0.3 m/s (0.2 to 0.4 m/s) in the relaxation group, resulting in an adjusted treatment effect of 0.1 m/s (0.0 to 0.2 m/s; P=0.23, primary efficacy outcome). The adjusted mean change in Barthel index score three months after stroke was 30 points (95% confidence interval 24 to 36) in the aerobic physical fitness training group compared with 30 points (23 to 36) in the relaxation group, resulting in an adjusted treatment effect of 0 (95% confidence interval −5 to 5; P=0.99, primary efficacy outcome). Figure 3 shows the change in maximal walking speed and Barthel index score during the trial.

In the per protocol analysis, the mean change in maximal walking speed was 0.4 m/s (95% confidence interval 0.3 to 0.5 m/s) in the aerobic physical fitness training group and 0.3 m/s (0.2 to 0.4 m/s) in the relaxation group, resulting in a treatment effect of 0.1 m/s (95% confidence interval −0.1 to 0.2 m/s). The mean increase in Barthel index score after three months was similar in both groups: aerobic physical fitness training 32 (95% confidence interval 28 to 37) versus relaxation 31 (27 to 35); treatment effect 1 (95% confidence interval −4 to 6). Figure 4 shows the results for the prespecified subgroup analyses. Exploratory subgroup analysis for maximal walking speed suggested a greater treatment effect in women than in men: 0.3 (95% confidence interval 0.1 to 0.5) v 0.0 (−0.2 to 0.1); P=0.01 for interaction. Figure 5 and supplementary figure S2 show the results for subgroup analyses of continuous variables using splines.

All secondary analyses were done in an exploratory framework. Overall, no substantial differences in maximal walking speed or Barthel index score between groups were observed at the end of intervention or at follow-up six months after stroke (table 3).
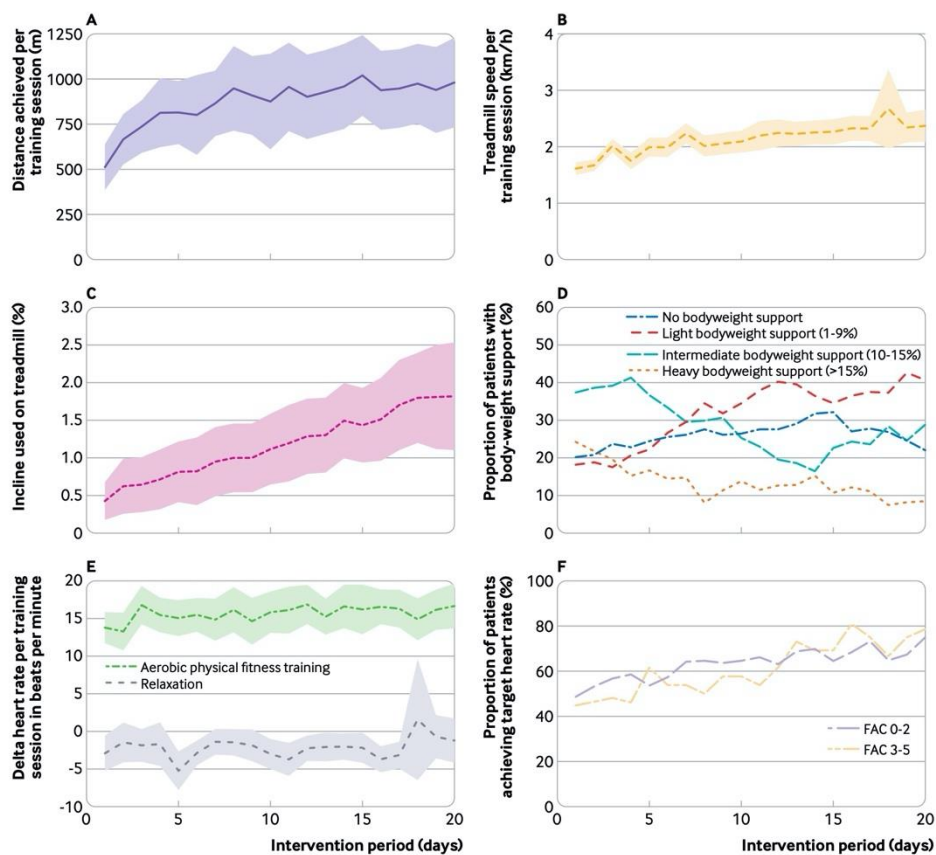
Fig 2 | Progression of training modalities during intervention period. (A) Distance (m) achieved (only available for participants who used treadmill). (B) Walking speed (km/h) reached on treadmill. (C) Change in incline (%) on treadmill (only available for participants who used treadmill). (D) Proportion of participants with different levels of bodyweight support over time. (E) Mean change in heart rate by group measured before and after intervention sessions during intervention period. (F) Proportion of participants who achieved their target heart rate during training. Participants are grouped by baseline functional ambulation category (FAC) score of 0-2 and 3-5 (higher scores indicate less dependency)

The six minute walk test showed a mean adjusted difference of 27 m (95% confidence interval 0 to 54 m) in favour of aerobic physical fitness training compared with relaxation at three months after stroke (adjusted for baseline, age, sex, and functional ambulation category). Other exploratory secondary analyses did not show substantial differences between study groups three months after stroke. At follow-up six months after stroke, the relaxation group showed greater improvements in the resistance to passive movement scale sum score (treatment effect 2.6 (95% confidence interval 0.6 to 4.5)) and the Rivermead mobility index subtest arm (treatment effect −1.1 (−2.0 to 0.0)) compared with aerobic physical fitness training (table 3).

The aerobic physical fitness training group had a higher rate of serious adverse events (n=22) than the relaxation group (n=9) at three months after stroke (incidence rate ratio 1.81, 95% confidence interval 0.97 to 3.36). Fourteen participants in the aerobic physical fitness training group were admitted to acute hospitals compared with five in the relaxation group (2.53, 0.91 to 7.04). Recurrent strokes occurred in eight participants in the aerobic physical fitness training group and in three participants in the relaxation group

Table 2 | Results for primary efficacy outcome of change in maximal walking speed and Barthel index score from baseline to three months after stroke by aerobic physical fitness training or relaxation sessions (control group)

Primary outcomes	Aerobic physical fitness training (n=105)	Relaxation sessions (n=95)	Adjusted treatment effect*	P value
Mean (95% CI) maximal walking speed (m/s)	0.4 (0.3 to 0.4)	0.3 (0.2 to 0.4)	0.1 (0.0 to 0.2)	0.23
Mean (95% CI) Barthel index score	30 (24 to 36)	30 (23 to 36)	0 (−5 to 5)	0.99

Analyses based on multiple imputation.
*Treatment effects were analysed using analysis of covariance mixed models with three month outcome as dependent variable adjusted for baseline and additionally adjusted for sex, study centre, and functional ambulation category.
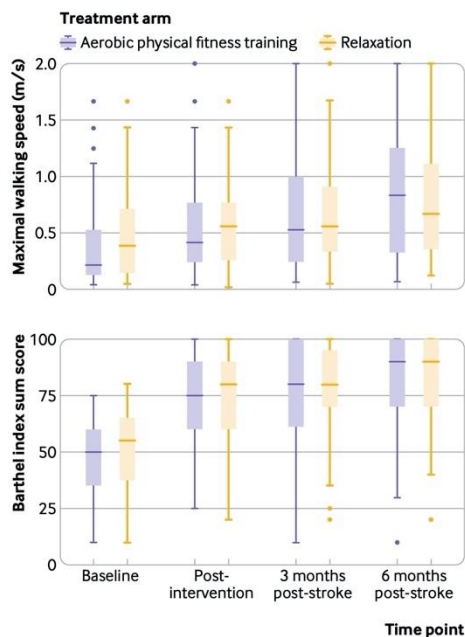
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Fig 3 | Boxplot showing medians (interquartile ranges) for maximal walking speed assessed by 10 m walk test (top panel), and Barthel index score (bottom panel) for each study visit and intervention group. Data are based on measurements without multiple imputation. Number of participants at each scheduled study visit was: baseline (n=105 in aerobic physical fitness training group, n=95 in relaxation group:), post-intervention (n=87, n=85), three months post-stroke (n=89, n=77), and six months post-stroke (n=80, n=65). Dots represent outliers

(2.41, 0.64 to 9.10). No serious adverse events occurred during intervention sessions. Self reported falls during the intervention period occurred more often in the aerobic physical fitness training group (2.34, 1.26 to 4.34) and self reported dizziness occurred more often in the relaxation group (0.33, 0.12 to 0.90, table 4).

Discussion
In adults with moderate to severe subacute stroke receiving standard inpatient rehabilitation therapy, a four week intervention of additional aerobic physical fitness training was not superior to a control intervention based on relaxation in improving activities of daily living and maximal walking speed three months after stroke. Analysis of safety showed an increased risk of falls during the treatment period and suggested a higher number of acute hospital admissions and recurrent strokes in participants randomised to aerobic physical fitness training. For clinical practice, the results of this pragmatic trial[26] do not support the use of aerobic physical fitness training in moderately or severely affected adults in the subacute phase of stroke.

Comparison with other studies
The treatment effects in mobility outcomes observed in the current study are in line with results reported in the latest Cochrane Collaboration meta-analysis of physical fitness trials after stroke[6] and randomised trials of circuit class training interventions.[27 28] In our trial, however, neither the intention-to-treat analysis nor the per protocol analysis showed a substantial difference in maximal walking speed between study groups. This finding contrasts with that of previously published trials reporting beneficial effects of fitness training on maximal walking speed.[29-31] Interventions in these previous trials were compared with usual care only, however, and the reported improvements were in smaller cohorts comprising participants with late subacute or chronic stroke and less severe disability. As recently endorsed by the Stroke Recovery and Rehabilitation Roundtable taskforce, trials should focus on the early subacute phase of stroke (days 7-90 after stroke)[10]; in the present trial more than 99% of participants were recruited in this subacute phase. This phase is thought to be a critical time for neuroplasticity[32] and therefore might serve as an important period for rehabilitation to harness endogenous neural repair. Despite early enrolment of participants to our trial, we were not able to show enhanced treatment effects. Interestingly, a recent meta-analysis of randomised controlled trials on bodyweight supported treadmill training after stroke concluded that participants who walked independently (functional ambulation category score >2) improved in walking speed and walking endurance, whereas those who were not able to walk independently (score 0-2) did not improve with treadmill training.[33] This fact could explain why our trial failed to show a statistically significant improvement in primary outcome measures, given that about 75% of the study cohort had functional ambulation category scores between 0 and 2 at baseline. However, no interaction between functional ambulation category score and treatment effect was observed in the respective subgroup analysis (fig 4 and supplementary figure S2). No treatment effect was observed with activities of daily living three months after stroke, which contrasts with the findings of smaller, previous trials including participants in the early and late subacute stage of stroke.[12 13] Until now, evidence that physical fitness reduces disability after stroke has been inconclusive for activities of daily living, and positive effects on disability after stroke could primarily derive from improvements in mobility.[6] Aerobic physical fitness training compared with relaxation had no beneficial effect on scores on the Barthel index, functional ambulation category, or modified Rankin scale (see tables 2 and 3). Current outcome measures of activities of daily living differ in sensitivity, and the Barthel index might not be sensitive enough to capture small but clinically meaningful differences in activities of daily living related to aerobic training after stroke. Exploratory analyses of secondary outcome measures suggested small beneficial effects in fitness measures for participants randomised to aerobic physical fitness training at three months after stroke, as reflected in the six minute walk test (see table 3). This was a secondary exploratory finding, however, and cannot be used for inferences on treatment effects.
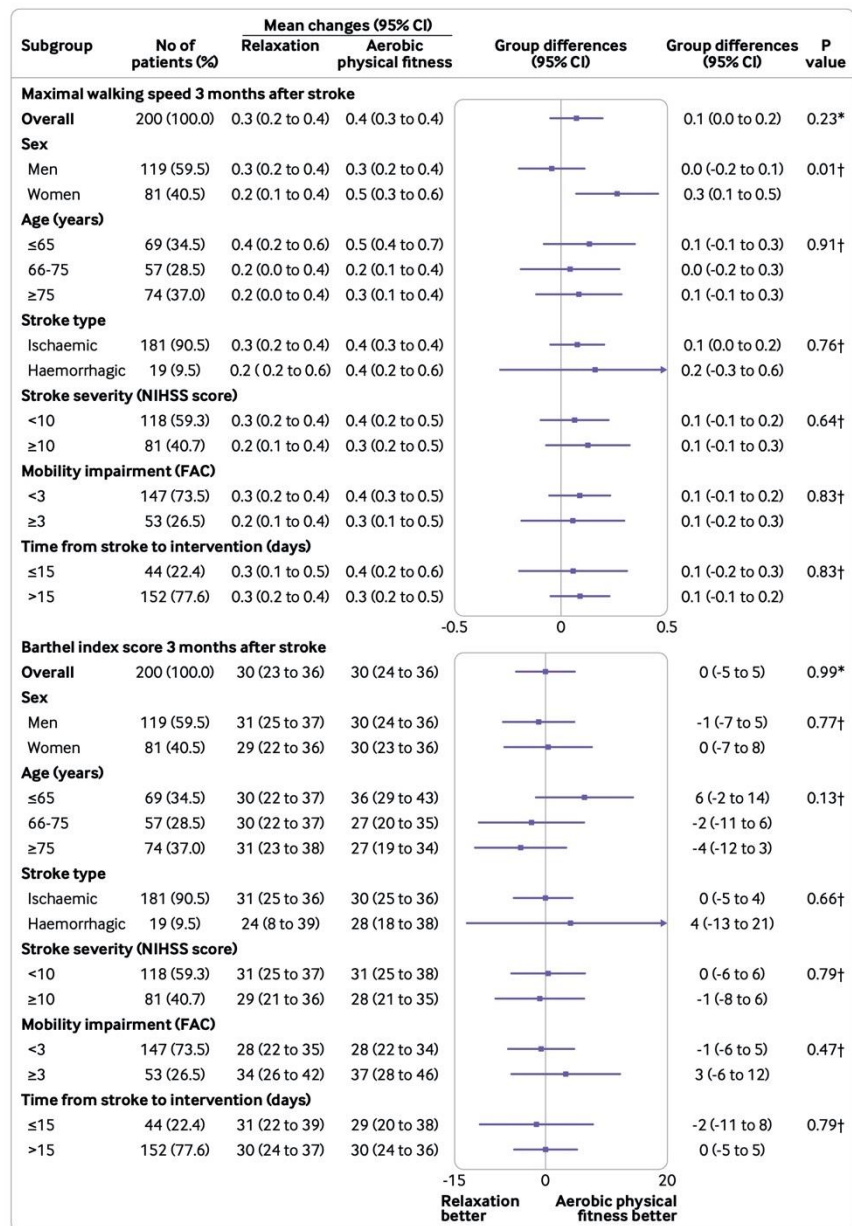
Fig 4 | Prespecified subgroup analyses. Forest plots display maximal walking speed and Barthel index scores. Results are based on multiple imputation. No data were available for time from stroke to intervention for four participants who were excluded at screening. National Institutes of Health Stroke scale (NIHSS) score was missing for one participant owing to missing hospital chart. FAC=functional ambulation category. *P value for primary outcome measure. †P values for age×group interaction

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Fig 5 | Subgroup analyses of continuous variables using splines. Differences in maximal walking speed and Barthel index scores (follow-up three months after stroke-baseline) as a function of age and National Institutes of Health stroke scale (NIHSS) score at days 3-5 after stroke

Thus, adequately powered trials need to confirm whether bodyweight supported, treadmill based, aerobic physical fitness training improves measures of endurance at a clinically important level in this stroke population.

No safety concerns were reported for aerobic physical fitness training in the early subacute phase in two smaller previous studies comprising survivors of mild to moderate stroke.[34][35] However, in the large randomised controlled LEAPS trial, the proportion of participants with multiple falls was higher in the early locomotor training group (starting two months after stroke) compared with the late locomotor training group (starting six months after stroke).[11] Similarly, in the current trial, falls occurred more often in the aerobic physical fitness training group than in the

Table 3 | Results for secondary outcome measures by aerobic physical fitness training or relaxation sessions (control group)

Secondary outcomes by groups	Baseline: aerobic physical fitness training (n=105), relaxation sessions (n=95)	Post-intervention: aerobic physical fitness training (n=87), relaxation sessions (n=85)	Follow-up 3 months: aerobic physical fitness training (n=89), relaxation sessions (n=77)	6 months: aerobic physical fitness training (n=79), relaxation sessions (n=65)	Treatment effect (95%CI)* Post-intervention	3 months follow-up	6 months follow-up
Median (interquartile range); mean (SD) maximal walking speed (m/s)†							
Aerobic training	0.2 (0.1-0.5); 0.4 (0.4)	0.4 (0.2-0.8); 0.6 (0.5)	0.6 (0.3-1.1); 0.8 (0.6)	0.8 (0.3-1.3); 0.9 (0.8)	0.03 (−0.10 to 0.16)	Primary outcome	0.09 (−0.04 to 0.22)
Relaxation	0.3 (0.1-0.7); 0.5 (0.4)	0.6 (0.3-0.8); 0.6 (0.6)	0.6 (0.4-0.9); 0.8 (0.7)	0.8 (0.4-1.3); 0.9 (0.9)			
Median (interquartile range); mean (SD) Barthel index score							
Aerobic training	50 (35-60); 47 (16)	75 (60-90) 73 (21)	80 (61-100); 77 (22)	90 (70-100); 82 (20)	0 (−4 to 5)	Primary outcome	−1 (−6 to 3)
Relaxation	55 (35-65); 49 (17)	80 (60-90); 75 (22)	80 (70-95); 79 (19)	90 (70-100); 84 (18)			
Median (interquartile range); mean (SD) 6 minute walk distance (m)‡							
Aerobic training	75 (32-160); 107 (110)	145 (85-245); 175 (126) (n=81)	165 (90-300); 201 (153) (n=85)	220 (110-350); 239 (152) (n=77)	19 (−8 to 46)	27 (0 to 54)	26 (−1 to 53)
Relaxation	120 (39-205); 139 (113)	179 (91-244); 185 (115) (n=78)	180 (110-263); 203 (128) (n=71)	208; (114-323) 233 (149) (n=64)			
Mean (SD) Rivermead mobility index score							
Aerobic training	5 (3)	8 (4)	9 (4)	11 (4)	0.2 (−0.6 to 1.0)	0.3 (−0.5 to 1.1)	0.0 (−0.8 to 0.8)
Relaxation	6 (3)	9 (4)	9 (4)	11 (4) (n=65)			
Median (interquartile range) modified Ranking scale score							
Aerobic training	4 (4-4)	4 (3-4)	3 (2-4) (n=90)	3 (2-4)	2.0 (0.6 to 6.9)§	0.8 (0.2 to 2.5)§	1.1 (0.3 to 3.6)§
Relaxation	4 (3-4)	3 (3-4)	3 (3-4) (n=78)	3 (2-4)			
Median (interquartile range) actigraphy (steps/day)¶							
Aerobic training	3263 (1815-5515); (n=97)	4758 (2910-7056); (n=70)	4215 (2042-6399); (n=77)	4284 (2193-7308); (n=63)	−555 (−1486 to 375)	−539 (−1467 to 394)	−566 (−1497 to 365)
Relaxation	3503 (1949-6328); (n=88)	5183 (2945-7876); (n=75)	5160 (3194-7980); (n=69)	6105 (3404-7904); (n=53)			
Median (interquartile range) step length (m)**							
Aerobic training	0.31 (0.23-0.44); (n=99)	0.39 (0.30-0.51); (n=85)	0.43 (0.29-0.56); (n=88)	0.50 (0.35-0.63); (n=78)	0.04 (−0.01 to 0.07)	0.03 (−0.02 to 0.07)	0.03 (−0.01 to 0.07)
Relaxation	0.39 (0.29-0.48); (n=93)	0.40 (0.30-0.50); (n=83)	0.42 (0.34-0.56); (n=76)	0.47 (0.33-0.64); (n=64)			
No (%) used walking aid							
Aerobic training	69 (75) (n=92)	57 (71) (n=80)	55 (63) (n=88)	48 (60) (n=79)	0.46§ (0.10 to 2.19)	0.29§ (0.06 to 1.41)	0.30§ (0.06 to 1.57)
Relaxation	60 (78) (n=77)	59 (76) (n=78)	52 (72) (n=72)	42 (68) (n=62)			
Median (interquartile range) step cadence (steps/min)††							
Aerobic training	53 (29-91); (n=99)	68 (46-102); (n=84)	91 (54-116); (n=88)	100 (59-128); (n=78)	2 (−7 to 12)	6 (−4 to 15)	3 (−7 to 12)
Relaxation	70 (36-92); (n=93)	80 (57-99); (n=83)	90 (65-113); (n=76)	98 (68-126); (n=64)			
Median (interquartile range) box and block test, impaired hand/non-impaired hand‡‡							
Aerobic training	0 (0-31) / 46 (34-54) (n=100)	12 (0-38) / 48 (40-58) /	19 (0-39) / 53 (40-62)	27 (0-44) / 54 (42-62)	1 (−3 to 6)	1 (−3 to 5)	−1 (−6 to 3)
Relaxation	2 (0-23) / 45 (35-52) (n=89)	12 (0-34) / 46 (39-56) /	23 (0-35) / 45 (38-56)	28 (3-43) / 50 (39-60)			
Mean (SD) Rivermead mobility index score: subtest arm							
Aerobic training	5 (5)	6 (6)	6 (6)	8 (6)	−0.7 (−1.6 to 0.2)	−0.9 (−1.8 to 0.0)	−1.1 (−2.0 to 0.0)
Relaxation	5 (5)	7 (5)	8 (5)	9 (5)			
Mean (SD) medical research council scale, sum score over six items§§							
Aerobic training	19 (8) (n=104)	22 (7) (n=86)	22 (6)	23 (7)	0.3 (−0.8 to 1.4)	−0.3 (−1.4 to 0.8)	−0.2 (−1.3 to 0.9)
Relaxation	20 (8)	22 (7) (n=85)	23 (7)	24 (5)			
Mean (SD) resistance to passive movement scale sum score							
Aerobic training	4 (5)	6 (6)	8 (9) (n=88)	11 (14)	0.9 (−1.0 to 2.9)	1.6 (−0.3 to 3.6)	2.6 (0.6 to 4.5)
Relaxation	4 (5)	5 (6)	6 (7) (n=77)	9 (9)			
Median (interquartile range) functional ambulation category score¶¶							
Aerobic training	2 (1-2)	3 (2-3) (n=56)	3 (2-4) (n=52)	4 (2-5) (n=47)	0.3 (0.1 to 1.6)§	0.9 (0.2 to 5.0)§	1.1 (0.2 to 6.2)§
Relaxation	2 (1-3)	3 (2-4) (n=57)	3 (2-4) (n=44)	3 (3-4) (n=33)			
Median (interquartile range) gait energy cost (ml/kg⁻¹/m⁻¹)***							
Aerobic training	0.8 (0.4-1.2) (n=54)	0.4 (0.3-0.6) (n=51)	0.4 (0.3-0.6) (n=49)	0.3 (0.2-0.5) (n=35)	−0.1 (−0.2 to 0.0)	−0.1 (−0.2 to 0.0)	−0.1 (−0.2 to 0.0)
Relaxation	0.4 (0.3-0.7) (n=46)	0.4 (0.3-0.5) (n=41)	0.4 (0.3-0.6) (n=41)	0.4 (0.3-0.5) (n=32)			
Mean (SD) quality of life (EQ-5D-5L) index score†††							
Aerobic training	0.5 (0.3) (n=104)	0.7 (0.3) (n=87)	0.7 (0.3) (n=87)	0.7 (0.3)	0.04 (−0.04 to 0.11)	0.03 (−0.05 to 0.11)	0.0 (−0.08 to 0.08)
Relaxation	0.5 (0.3) (n=93)	0.7 (0.3) (n=82)	0.6 (0.3) (n=77)	0.7 (0.3)			
Mean (SD) depression (CES-D) sum score‡‡‡							
Aerobic training	10 (7) (n=85)	9 (6) (n=72)	10 (7) (n=71)	8 (7)	−1 (−3 to 1)	0 (−2 to 2)	0 (−2 to 1)
Relaxation	10 (5) (n=72)	10 (5) (n=67)	10 (6) (n=64)	9 (5)			

(continued)

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Table 3 | Continued

Secondary outcomes by groups	Baseline: aerobic physical fitness training (n=105), relaxation sessions (n=95)	Post-intervention: aerobic physical fitness training (n=87), relaxation sessions (n=85)	Follow-up 3 months: aerobic physical fitness training (n=89), relaxation sessions (n=77)	6 months: aerobic physical fitness training (n=79), relaxation sessions (n=65)	Treatment effect (95%CI)* Post-intervention	3 months follow-up	6 months follow-up
Mean (SD) sleep (PSQI) sum score§§§							
Aerobic training	4 (3) (n=96)	5 (4) (n=83)	6 (3) (n=84)	5 (4) (n=77)	−1 (−2 to 0)	−1 (−1 to 0)	−1 (−1 to 0)
Relaxation	5 (3) (n=88)	6 (4) (n=78)	6 (4) (n=74)	5 (3) (n=60)			
Median (interquartile range) cognition (MOCA) sum score¶¶¶							
Aerobic training	24 (21-27) (n=104)	26 (22-28) (n=86)	25 (22-28) (n=89)	26 (24-29)	0 (−1 to 1)	−1 (−1 to 1)	0 (−1 to 1)
Relaxation	24 (17-26) (n=94)	25 (19-27) (n=84)	25 (21-28) (n=75)	26 (21-28) (n=64)			
Median (interquartile range) cognitive processing speed (TMT A) (sec)****							
Aerobic training	70 (52-122) (n=104)	56 (42-120) (n=85)	51 (37-84)	52 (33-69)	5 (−10 to 20)	7 (−7 to 22)	1 (−14 to 16)
Relaxation	85 (60-197) (n=94)	64 (47-123) (n=84)	67 (43-102)	60 (42-82)			
Median (interquartile range) executive functioning (TMT B) (sec)††††							
Aerobic training	222 (127-301) (n=104)	97 (93-301) (n=85)	139 (88-301)	125 (78-216)	−5 (−21 to 11)	−1 (−17 to 16)	−6 (−22 to 10)
Relaxation	301 (157-301) (n=93)	218 (134-301) (n=84)	166 (110-301)	150 (92-301)			
Mean (SD) word fluency (RWT) sum score‡‡‡‡							
Aerobic training	38 (19) (n=104)	-	43 (19) (n=86)	-	-	1 (−2 to 3)	-
Relaxation	34 (18) (n=92)	-	40 (18) (n=73)	-			

Analyses are based on mixed models analysis of covariance (adjusted for baseline value, age, sex, functional ambulation category, and centre heterogeneity). Estimates are based on three level mixed models and multiple imputation (n=600 measures, 200 participants, six study centres) positive values favour aerobic physical fitness training. Missing values were imputed by multiple imputation, except for modified Ranking scale score, use of walking aids, and functional ambulation category (imputation process was done separately for treatment groups). If the number of valid data points differs for specific variables, the number of available data points is listed in brackets. Data missing due to missing at random are imputed (see supplementary appendix).
*Secondary outcomes are exploratory and not meant for hypothesis testing. P values are therefore not reported.
†21 participants were unable to walk 10 m to assess maximal walking speed—values were therefore imputed using single value imputation by taking half of lowest value of total cohort.
‡17 participants were unable to walk for entire time of six minutes—for those participants distance walked up to stopping is used. 28 participants were in poor physical condition and could not do the test—values were therefore imputed, using single value imputation by taking half of lowest value of total cohort.
§Difference between groups at three months after stroke presented as odds ratios (95% confidence intervals, odds ratio >1 favours aerobic training intervention. Calculations not adjusted for centre heterogeneity. For walking aids odds ratio >1 means dependence on walking aids in aerobic training group. Table 2 shows results for primary efficacy outcomes.
¶15 baseline actigraphy values were missing due to logistic reasons. Data are assumed to be missing completely at random.
**Missing values in step length are due to implausible number of steps within 10 m gait assessment, or 10 m walk test not completed.
††Missing values in step cadence are due to implausible number of steps within 10 m gait assessment, or 10 m walk test not completed.
‡‡11 participants showed no initial motor impairment and are excluded from analysis.
§§One baseline value on medical research council scale is missing at random in aerobic group.
¶¶Functional ambulation category was initially assessed at baseline only and not at follow-up; therefore some follow-up values are missing.
***Spirometry data are missing mostly due to technical issues. Baseline values are available for 100 participants (n=54 aerobic training, n=46 relaxation).
†††Six baseline values for EuroQol quality of life questionnaire (EQ-5D-5L) index score were missing for various reasons (aphasia, fatigue, understanding difficulties).
‡‡‡19 baseline values for Center for Epidemiologic Studies depression scale (CES-D) sum score were missing owing to aphasia, fatigue, understanding difficulties, not able to respond to question. 24 data points had to be excluded because participants fulfilled criteria for unreliable data (lie, criteria ≤28).
§§§16 baseline values for Pittsburgh sleep quality index (PSQI) sum score were missing owing to aphasia, fatigue, and understanding difficulties.
¶¶¶Two baseline values for Montreal cognitive assessment (MOCA) sum score were missing owing to fatigue.
****Two baseline values for trail making test (TMT) part A were missing owing to poor vision and fatigue.
††††Three baseline values for trail making test (TMT) part B were missing owing poor vision, fatigue, and failure of time recording by assessor.
‡‡‡‡The Regensburger Wortflüssigkeitstest (RWT) was only assessed at baseline and at three months' follow-up. Four baseline values for RWT were missing owing to severe aphasia.

relaxation group during the intervention period. In contrast, participants in the relaxation group reported dizziness more often than participants in the aerobic physical fitness training group (see table 4). People with stroke are at increased risk for repeat falls, and fall prevention programmes are needed.[36] Hence, and in opposition to current guideline recommendations,[7] treadmill based aerobic physical fitness training should be administered with caution early after moderate to severe stroke. A Very Early Rehabilitation Trial after stroke (AVERT) mobilised participants within 24 hours after stroke and found a worse outcome after three months, but no differences were observed in walking related outcome measures.[37] Although AVERT, LEAPS, and the present trial differ in terms of study population, timing after stroke, and type of intervention, these studies did not replicate the previously reported beneficial effects of specific rehabilitation interventions in small cohorts, while at the same time detecting a larger number of relevant adverse events and serious adverse events.

Strengths and limitations of this study

This is a randomised controlled, multicentre stroke rehabilitation trial assessing the efficacy and safety of an aerobic, bodyweight supported fitness intervention compared with relaxation in adults with subacute stroke. Compared with previous post-stroke fitness trials,[6] our trial recruited a substantially larger sample size comprising adults with moderate to severe stroke and assessed a broad spectrum of patient centred outcome measures. The study does, however, have limitations. Firstly, recruitment took place between days 5 and 45 after stroke, therefore variations in early neurological recovery could have increased differences in outcome measures and might have affected current conclusions. However, the impact of the additionally induced variations is limited because almost all

participants were recruited within the time window of early subacute stroke,[10] and subgroup analyses (see fig 4) could not detect differences in treatment effects for participants receiving the intervention early after stroke (<2 weeks) compared with late after stroke (>2 weeks). Secondly, despite random allocation, the aerobic physical fitness training group was more severely affected at baseline, but we adjusted for baseline values in statistical analyses. Thirdly, findings are only applicable to moderately to severely affected adults with subacute stroke and are not generalisable to the stroke population, especially not to people with chronic stroke. Fourthly, less than 4% of the screened adults with stroke were included in the trial, and the severity of impairment might have resulted in a substantial proportion of participants terminating the intervention prematurely; a limitation often observed in early stroke rehabilitation trials.[9 11 38] Fifthly, the intervention period of four weeks could have been too short to show additional benefits compared with a relaxation control intervention; and the lack of a control arm for usual care only limits the understanding of the treatment effects of relaxation therapy.

Clinical implications and future research

Our trial provides evidence that an aerobic physical fitness training intervention with bodyweight support cannot be generally endorsed in adults after subacute stroke and should be administered with caution when applied early after moderate or severe stroke. Carers should closely monitor people with stroke for recurrent cardiovascular events and provide additional support after training to prevent falls. Despite these findings, aerobic physical fitness could still be an invaluable part of stroke rehabilitation—for example, in people with chronic stroke or mildly affected with subacute stroke.

Based on our results, trials should investigate if longer intervention periods are necessary to capture major changes in activities of daily living, assess the treatment effects of relaxation programmes compared with usual care, and recruit participants at fixed time points after stroke to reduce variance in outcome measures.[10 39] Future trials might also evaluate whether the observed sex specific difference in change of maximal walking speed or the effects on endurance measures can be replicated.

Table 4 | Safety outcomes by aerobic physical fitness training or relaxation session (control group)

Events	Aerobic physical fitness training (n=105)	Relaxation sessions (n=95)	Total cohort (n=200)	Incidence rate ratio (95% CI)*
Serious adverse events† (from baseline to three months after stroke)				
Median (interquartile range) follow-up (days)	68 (56-78)	69 (54-77)	66 (56-78)	
Total No	22	9	31	
Incidence rate/100 person months (95% CI)	13.19 (9.22 to 18.86)	7.28 (4.39 to 12.08)	10.38 (7.75 to 13.90)	1.81 (0.97 to 3.36)
Cardiovascular event	0	0	0	-
No with recurrent stroke	8	3	11	-
Incidence rate/100 person months (95% CI)	3.52 (1.76 to 7.03)	1.46 (0.47 to 4.52)	2.54 (1.41 to 4.58)	2.41 (0.64 to 9.10)
No of hospital admissions	14	5	19	-
Incidence rate/100 person months (95% CI)	6.15 (3.64 to 10.39)	2.43 (1.01 to 5.83)	4.39 (2.80 to 6.87)	2.53 (0.91 to 7.04)
No of deaths	0	1	1	-
Incidence rate/100 person months (95% CI)	-	0.49 (0.07 to 3.45)	0.23 (0.03 to 1.64)	0.30 (0.01 to 7.42)‡
Self reported adverse events§ (during intervention period)				
Median (interquartile range) follow-up time (days)	33 (29-36)	22 (28-36)	33 (29-36)	
No of falls	36	14	50	-
Incidence rate/100 person months (95% CI)	32.40 (23.37 to 44.92)	13.84 (8.19 to 23.36)	11.8 (8.9 to 15.5)	2.34 (1.26 to 4.34)
No of fractures	0	0	0	-
No with pain	68	44	112	-
Incidence rate/100 person months (95% CI)	61.20 (48.25 to 77.62)	43.49 (32.36 to 58.44)	26.1 (21.7 to 31.4)	1.41 (0.96 to 2.06)
No with fatigue	29	21	50	-
Incidence rate/100 person months (95% CI)	26.10 (18.14 to 37.56)	20.76 (13.53 to 31.83)	23.55 (17.85 to 31.07)	1.26 (0.72 to 2.20)
No with dizziness	5	14	19	-
Incidence rate/100 person months (95% CI)	4.50 (1.87 to 10.81)	13.84 (8.19 to 23.36)	8.95 (5.71 to 14.03)	0.33 (0.12 to 0.90)
Other	8	4	12	-
Incidence rate/100 person months (95% CI)	7.20 (3.60 to 14.40)	3.95 (1.48 to 10.53)	5.65 (3.21 to 9.95)	1.82 (0.55 to 6.05)

*Calculated using Poisson regression.
†Serious adverse event can belong to more than one category (eg, recurrent stroke and hospital admission). Thus total number of participants with a serious adverse event does not equal sum of individual categories of serious adverse events.
‡Calculated using penalised maximum likelihood logistic regression (firthlogit command in stata).
§Adverse events can occur multiple times in a participant. Time interval ranges from baseline to follow-up at three months after stroke or until last observation, if participant dropped out.

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Conclusion and policy implications

A four week intervention of a bodyweight supported, treadmill based, aerobic physical fitness training in adults in the subacute phase of moderate to severe stroke is not superior to relaxation sessions with regard to maximal walking speed and activities of daily living. The risk of falls was higher in participants randomised to aerobic physical fitness training. Compared with current guideline recommendations,[7] these results do not appear to support the use of aerobic bodyweight supported fitness training in this stroke population.

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Supplementary material: inclusion and exclusion criteria
Supplementary material: appendix

Inclusion and Exclusion Criteria of the PHYS-STROKE trial.

INCLUSION CRITERIA	
1.	**Diagnosis of stroke (inclusion within 5-45 days after stroke onset); ischemic or haemorrhagic (cortical, subcortical, brainstem), as determined by initial MRI/CT scan of the brain)**
2.	Age $\geq$ 18 years
3.	Able to sit unsupported (i.e. without holding onto supports such as the edge of the bed), with feet supported, for at least 30 seconds
4.	Barthel-Index $\leq$ 65 at inclusion
5.	Considered able to perform aerobic exercise, as determined by responsible physician
6.	Provision of written informed consent
EXCLUSION CRITERIA	
1.	Patient considered unable to comply with study requirements
2.	Stroke due to intracranial haemorrhage primarily due to bleeding from ruptured aneurysm or arteriovenous malformation
3.	Progressive stroke
4.	Unable to perform the required exercises due to a) medical, b) musculo-skeletal, or c) neurological problems (for details see below, 4a-c)
4a.	Medical problems: unstable cardiovascular condition, or other serious cardiac conditions (e. g., New York Heart Association criteria for Class IV heart disease, hospitalization for myocardial infarction or heart surgery within 120 days, severe cardiomyopathy or documented serious and unstable cardiac arrhythmias)
4b.	Musculoskeletal problems: restricted passive range of motion in the major lower limb joints (i.e. an extension deficit of > 20° for the affected hip or knee joints, or a dorsiflexion deficit of > 20° for the affected ankle)
4c.	Neurological problems: severity of stroke-related deficits
5.	Required help of at least 1 person to walk before stroke due to neurological (e. g., advanced Parkinson's disease, Amyotrophic Lateral Sclerosis, Multiple Sclerosis) or non-neurological co-morbidities (e. g. heart failure, orthopaedic problems)
6.	Life expectancy of less than 1 year as determined by responsible physician
7.	Drug or alcohol addiction within the last six months
8.	Significant current psychiatric illness defined as medication-refractory of bipolar affective disorder, psychosis, schizophrenia or suicidality.
9.	Current participation in another interventional trial

MRI = magnetic resonance imaging | CT = computer tomography.

Physical Fitness Training in Patients with Subacute Stroke (PHYS-STROKE) – a multicentre, randomised-controlled, endpoint-blinded trial

Supplementary Appendix

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Table of contents

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D Nabavi.

Medical Park Berlin Humboldtmühle, Berlin (*26.09.2013 – 27.10.2017; 76 / 75*): M Ebinger.

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Nave, Rackoll et al.; p.4

Supplementary Methods

Study Design

The study was designed to test the efficacy of an aerobic physical fitness intervention that can be administered in a clinical setting. Thus, trial interventions were directly applied at neurorehabilitation clinics next to usual care. Several aspects of the trial design follow principles of pragmatic trials. Accordingly, we have evaluated the PHYS-STROKE trial regarding the nine dimensions proposed by the Pragmatic–Explanatory Continuum Indicator Summary 2 (PRECIS-2) tool for assessing the level of pragmatism in a trial (1). As a result, PHYS-STROKE should be regarded as a rather pragmatic trial (see Figure S1).
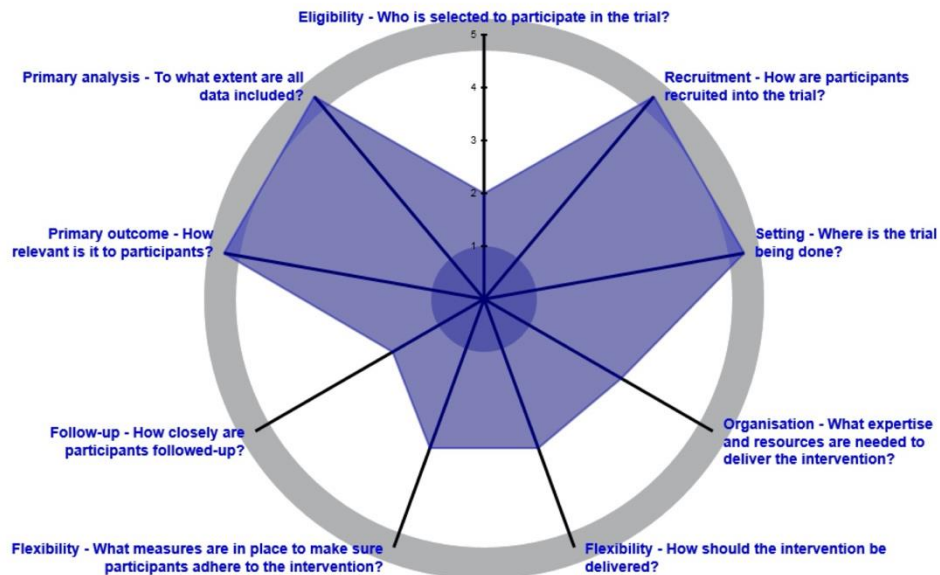
Figure S1: The Pragmatic–Explanatory Continuum Indicator Summary-2 (PRECIS-2) wheel of the PHYS-STROKE trial. Nine dimensions display the level of pragmatism of a trial with scores ranging from one (very explanatory) to five (very pragmatic).

Study protocol

The English version of the study protocol[1] was first published in the 'Trials' Journal:

Flöel A, Werner C, Grittner U, *et al. Physical fitness training in Subacute Stroke (PHYS-STROKE)--study protocol for a randomised controlled trial*. Trials 2014; 15: 45.

Study protocol: Amendments

In the course of the study the ethics committee of the Charité-Universitätsmedizin Berlin approved four amendments (02.09.2013; 23.04.2015; 15.11.2016; 21.08.2017). The following data were added:

Table S1: Amendments approved by the ethics committee of the Charité-Universitätsmedizin Berlin.

Amendment and Date of Votum	Changes	Reasons for changes
Amendment and Votum 23.04.2015	Seven day accelerometry, Freiburg questionnaire on physical activity (short version). Implementation of two new trial sites (Charité-Universitätsmedizin Berlin, Institute of Neurology and Vivantes Klinikum Neukölln, Berlin, Klinik für Neurologie).	At follow-up 3 months post stroke, most subjects are still in inpatient clinical care where daily variation of physical activity is limited. Variation of physical activity is greater in a home setting. Thus, a seven-day accelerometry is considered a more precise assessment at follow-up 6 months post stroke. The German Freiburg questionnaire on physical activity is validated in a broad age range in the German population and is capable of estimating daily energy expenditure. It is regarded as a valuable measure in addition to the accelerometry measurement to assess long-term effects of the intervention on physical activity. Two more trial sites are added as a consequence of difficulties in recruitment.
Amendment and Votum 15.11.2016	Audio recording of the Regensburg semantic and phonemic word fluency test.	To increase the explanatory power of the Regensburg semantic and phonemic word fluency test, audio recordings are necessary to distinguish temporal patterns (temporal cluster analysis) of word production.
Amendment and Votum 12.09.2017	Small administrative changes (no additional assessments).	The local PI changed from Dr. Flöel to Dr. Endres due to a change of the affiliation of Dr. Flöel.

Randomisation and masking

Randomisation used Functional Ambulation Category (FAC; dichotomised FAC≤3), centre, and age (dichotomised age at ≤65 years) as strata and was done in a 1:1 fashion. Each strata was organised in blocks of 10 subjects. The randomisation procedure was done by a clinician of each study site after the baseline visit was performed. The assignment was subsequently communicated to the treating therapists. The trial centre was not informed about patient allocation during the course of the trial.

Procedures: Care providers

Each study site assigned at least two therapists per intervention group to the trial, who were responsible for conducting the intervention. Care providers were supposed to be physiotherapists or sport therapists by training delivering the PHYS intervention and neuropsychologists or psychologists for the RELAX intervention respectively. A team member of the trial centre regularly visited each study site to discuss issues regarding the intervention and to control the adherence to adequate documentation. In addition, the trial monitoring controlled for every patient whether intervention documentation was adequate.

Procedures: Intervention manual

A written intervention manual was distributed to all trial therapists containing extensive description of the intervention and documentation procedures. Additionally, a web page with frequently asked questions was

maintained. A regular newsletter informed about specific procedures. A member of the trial centre visited each study site to discuss current issues with the treating therapists. The intervention manuals for both, PHYS and RELAX, are described in the following section:

PHYS – Intervention (experimental)

The aerobic physical fitness training (PHYS) intervention was designed to improve cardiorespiratory fitness while being functional in terms of applying locomotion therapy. The goal was to reduce deconditioning and enhance endogenous neuroplasticity following a cerebrovascular event. Cardiorespiratory load was targeted at 50–60 % of the estimated maximal heart rate to ensure an aerobic training in the lower range of recommended cardiorespiratory training intensities endorsed by the American College of Sports Medicine (ACSM) (2).

The target heart rate (THR) was calculated by care providers (therapists) at the beginning of the intervention period based on the formula: '180 – age'. The THR was adjusted with minus 10 beats in case of beta blocker intake. Before the first intervention session patients were allocated to receive training either in a gait trainer (FAC score 0 – 2) or on a body-weight supported treadmill (FAC 3 – 5). FAC was assessed by the therapists on a daily basis and subsequent training was administered using the respective allocated device.

Before each session, resting (pre-training) heart rate (HR) and blood pressure (RR) was assessed while the patient was still in a resting position. Training was only commenced if HR was below 180 bpm or systolic blood pressure below 200 mmHg, as recommended by the ACSM. Additionally, patients were equipped with a pulse belt for the therapist to constantly monitor the patient's HR.

Gait trainer:

The intervention manual refers to the Gait Trainer GT1, Reha-Stim, Berlin, Germany. General recommendations equally applied to other manufacturers. The patient was seated in the gait trainer following the manufacturer's manual. Attention was paid to the individual step length, and ensured that the hemiparetic arm was fixed to the handle bar in front of the patient. Body-weight support (BWS) was adjusted by the therapist, and only given as functionally needed. Training started with a three minute warm-up phase in which the patient started to practice at a low speed. After three minutes, the speed was increased until the THR was reached. After twenty minutes, the speed was reduced for another two minutes to reach a cool-down phase. Training at the THR was possible and constantly achieved due to the following adjustments of treatment modalities: decreasing BWS or increasing speed. The training aimed at delivering a constant cardiorespiratory load of 50 – 60 % maximum HR, represented by the THR. In addition patients who recover functionally were able to transition to treadmill based training if FAC was > 2.

Treadmill:

The intervention manual refers to the Multi-disk treadmill Callis, Model Therapie, SPRINTEX Trainingsgeräte GmbH, Kleines Wiesental, Germany. General recommendations equally applied to other manufacturers. The patient was supplied with the BWS irrespective of need to ensure safety. Body-weight support (BWS) was adjusted by the therapist only as much as functionally needed to allow proper trunk and limb alignment as well as weight shifting on the paretic limb. Patients started at the preferred walking speed over a warm-up period of three minutes. After three minutes, the speed was increased until the THR was reached. After twenty minutes of training, the speed was reduced for another two minutes to the individually preferred walking speed to reach a cool-down phase. Training at the THR was possible and constantly achieved due to the following adjustments of treatment modalities: increase of inclination, reduction of BWS, and increase of speed. The goal was to reduce BWS until 0 % was reached while still allowing for a proper weight bearing of the paretic limb with less than 15° of knee flexion. Patients were able to use handle bars, if needed. Therapists were advised to provide functional support by facilitating hip movement, support weight shifting, stabilizing of the knee, or setting of the paretic limb in case of paresis of the peroneus muscles. Recommendations were given for positioning of staff to ensure ergonomic posture of the therapists during foot placement. Patients were allowed to use orthoses if used also during usual care physiotherapy.

After the training the subject was seated on a chair to assess post-training HR, RR, and perceived rate of exertion during training on a visual analogue scale (0 – 10; 10 denotes highest possible exertion). After all equipment had been removed from the patient, he or she was asked if any adverse events occurred during the last 24 hours.

Nave, Rackoll et al.; p.7

Safety procedures:

Throughout the training, HR was monitored by the treating therapist. In case of a HR increase above 180 bpm training was stopped and a physician was contacted. Additionally training was stopped if the patient reported strong pain, constant dizziness, severe fatigue, or a strong urge to urinate. Patients were allowed to take short breaks, but therapists were advised to resume training as quickly as possible. The number and length of breaks were to be decreased over time. To ensure the patient was still practicing below the anaerobic threshold the talk test (3) was administered during each intervention session.

RELAX – Intervention (control)

The relaxation control intervention was designed according to the muscle relaxation after Jacobsen (4) in order to release from overall stress but to restrain from cardiorespiratory load.

A quiet room with either a comfortable chair or bed was used to administer the intervention. Before each session resting heart rate (HR) and blood pressure (RR) was assessed while the patient was still in a resting position. Additionally the patient was equipped with a pulse belt to constantly monitor HR. The patient was positioned to enable relaxation, and soft cushion support was provide to the limbs if needed.

The care provider (therapist) read the commands slowly to the patient (see Table S2). Additionally the patient was encouraged to focus on the feeling of warmth and heaviness in the addressed muscle group. Throughout the intervention session, the therapist ensured that the patient focused on the relaxation, but did not fall asleep.

After 25 minutes of relaxation training, HR and RR were documented and perceived rate of exertion assessed on a visual analogue scale (0 – 10; 10 denotes highest possible exertion). After all equipment had been removed from the patient, he or she was asked if any adverse events occurred during the last 24 hours.

The RELAX intervention was stopped if patients reported severe pain, strong fatigue, or the urge to urinate, and continued after the problem had been solved.

Nave, Rackoll et al.; p.8

Table S2: Routines for relaxation program.

No.	Phase	Instruction
1 Introduction		
1.1	R	Close your eyes, take several deep breaths, relax and feel how your body becomes loose and heavy. Try to not think about anything. Sense all the muscles in your body and try to relax as well as possible.
2 Hands and arms		
2.1	T	Now focus on your right hand. Make a fist and observe the tension. Hold your fist and feel the tension within your fist and the forearm.
2.2	R	And now relax. Let the fingers of your right hand go loose and pay attention to the difference.
2.3	T	And now do the same with your left hand. Make a fist while your body is relaxing and concentrate on the tension.
2.4	R	And now relax…
2.5	T	Clench now both fists and pay attention to your sensations.
2.6	R	And now release. Stretch your fingers and feel the relaxation. Progress in letting your hands and forearms go loose … Your hands are now warm and heavy.
3 Upper arm		
3.1	T	Now bend your elbows and strain your biceps. Pay attention on the sensation of tension.
3.2	R	And now stretch your arms again and focus on the difference. Notice how relaxation starts to spread.
3.3	T	Now extend your arms, and push them on the pad so that you have a strong sensation of tension in your upper arm. Feel the tension.
3.4	R	And now relax. Place your arms comfortably. Let the sensation of release spread. You feel a strong sensation of heaviness in your arms, while they relax.
4 Forehead and eyes		
4.1	T	Now pull your eyebrows towards your forehead so you feel wrinkles form on your forehead. Hold the tension.
4.2	R	And now relax your forehead and let it go loose and smooth again. Pay attention on how your skin becomes softer the more you relax. The entire skin relaxes.
4.3	T	Now pull your eyebrows together, so that a vertical wrinkle appears between your eyes. Pay attention to the sensation of tension.
4.4	R	And now relax again. Let your forehead go loose.
4.5	T	Squint your eyes tightly … and feel the tension
4.6	R	And now relax again. Let your eyes and your cheeks go loose and pay attention on the relaxation
5 Jaw muscles and lips		
5.1	T	Now push your teeth together. Pay attention to the tension which evolves in your jaw muscles.
5.2	R	Relax your jaw muscles. Leave your lips open just a little bit. Feel yourself relax.
5.3	T	Now tightly press your tongue against the roof of the mouth. Focus on the tension.
5.4	R	Release your tongue again and relax.
6 Throat		
6.1	T	Now turn your attention towards your neck muscles. Push the back of your head smoothly into the pad. While doing so your chin moves towards the breast bone. Tense your throat and neck muscles and focus on the tension.
6.2	R	And now release your neck. Place your head again in a comfortable position.
6.3	T	Bend your head to the front and push your chin against your chest as well as possible. And experience the sensation of tension.
6.4	R	Now place your head on the pad again and focus on the sensation of relaxation. Let the relaxation spread.
7 Neck and shoulders		
7.1	T	Now pull your shoulders towards your ears. Hold the tension.
7.2	R	Release your shoulders and experience how the neck and shoulders relax. Pay attention on how well all muscles release tension.
8 Shoulder blades		
8.1	T	Now pull your shoulder blades together and towards the back. Focus on how you experience the feeling of tension and where it is strongest.
8.2	R	And now let your shoulder blades return to the normal position and relax. Let the relaxation spread from your shoulders all the way towards the muscles of your back. Relax your neck, the throat, your jaw muscles and your entire face. Feel how a deep sensation of relief is spreading.
9 Chest		
9.1	R	Breath easy in and out. Pay attention on how your relaxation evolves with you breathing out. And while you exhale you feel the relief.
9.2	T	Now take a deep and strong breath and let it fill your lungs. Hold your breath for a short time. Pay attention to the sensation of tension.
9.3	R	… and let the air flow out by itself. Let your chest release. Feel the relief and continue to breath normally

Tension phase of muscles are supposed to be hold for 5 – 10 seconds while relaxation phase is supposed to yield for about 30 – 40 seconds. Only exception is for the chest in which the tension phase is much longer. T denotes tension, R denotes relaxation.

Nave, Rackoll et al.; p.9

If in any of the two intervention arms, a session was missed for any reason, study sites were asked to administer missed sessions until twenty sessions were reached. Patients were not allowed to miss training on more than five consecutive days.

Procedures: Protocol adherence

To achieve a standardised intervention regimen, therapists of study centres were trained in the application of the intervention procedures (instructor: Daniela Krohne) and in the documentation of daily intervention diaries following GCP-guidelines (instructor: Regina Schlieder). Quality checks of the intervention diaries were done with every monitoring visit. Additionally, regular visits were conducted at study sites by a member of the trial coordinating centre (Torsten Rackoll) to discuss the enrolment progress and answer inquiries concerning the intervention. Intervention diaries of both groups documented the time of attention of therapists, time spent in the active phase of each session (core intervention), heart rate, and blood pressure before and after the intervention as well as ratings of perceived exertion and adverse events that occurred in the course of the last 24 hours.

Procedures: Standard Care

Standard care was delivered following the German guidelines for neurorehabilitation after stroke (www.bar-frankfurt.de). Neurorehabiliation in Germany is categorized into several phases depending on the medical status of the patient and is organized as follows:

Phase B: Early rehabilitation (Barthel-Index < 30 points or need of acute medical treatment)

Phase C: Continuing rehabilitation (Barthel-Index 30 – 65)

Phase D: Rehabilitation following inpatient treatment (Barthel-Index 65 – 100)

Besides activating nursing, standard of care in German neurorehabilitation centres consists of physiotherapy, occupational therapy, physical therapy, neuropsychological therapy, speech and facio-oral therapy (http://www.icd-code.de/ops/code/8-552.html). In patients with a BI below 30 points, 300 minutes of therapy sessions per day are standard. In patients with a BI >65 points, the administered therapy time depends on its type: For physiotherapy, at least 180 minutes per week are provided (https://www.deutsche-rentenversicherung.de/Allgemein/de/Inhalt/3_Infos_fuer_Experten/01_sozialmedizin_forschung/downloads/quali_rehatherapiestandards/Schlaganfall/rts_schlaganfall_download.html). In patients with a BI between 30 and 65, the therapy duration may vary at the discretion of the treating physician. Content of each therapeutic approach are defined by the treating therapist and may vary in between study centres.

Recommendations follow the guidelines of the American Heart Association Stroke Council (13). To assure equal administration of therapies we assessed the duration of therapies received per patient between baseline and follow-up three months after stroke. The length of therapy sessions per patient was recorded by the study site during the period of inpatient rehabilitation therapy. After discharge from the study site, patients documented subsequent outpatient rehabilitation therapies in minutes and presented all documentation at follow-up visits.

Outcomes: Assessments of Secondary Endpoints

All outcome measures were assessed using a standardised manual. Study assessors trained the ratings until they reached an agreement for all ratings in each assessment.

<u>6-min Walking Test (6-MWT):</u>

The distance walked in six minutes (6-minute Walking Test, 6-MWT) was assessed at each centre using hallways not used by other patients or personnel during the test. Thirty-five metres were marked with a clear start and ending mark. The 6-MWT was conducted after the 10-metre gait test, but with a resting period in between. Subjects were asked to walk in a speed in which they would be able to walk safely for six minutes without interruption, but to try to cover as much distance as possible. Patients used the same orthoses and / or walking aids during walking

which they used during standard rehabilitative care. An additional person from the assessment team secured every patient's safety and assisted with walking, if needed. The test commenced when subjects started to walk and ended after six minutes. Patients were instructed to turn after 35 metres. The distance walked after six minutes was marked by the assessor. Blood pressure was measured directly after the test and exhaustion was subsequently assessed using a visual analogue scale (zero [0] marking the least possible effort and ten [10] the maximal exhaustion).

Rivermead Mobility Index (RMI) and Rivermead Motor Assessment Subtest Arm:

The Rivermead Mobility Index assesses functional mobility with 15 tasks in increasing difficulty. Patients were instructed to perform each task starting with the least challenging one. Each patient was allowed three attempts per task. If a patient was not able to perform one test, he or she was allowed to try the next, more challenging one. The assessor gave precise instructions and demonstrated the task, if instructions were not understood correctly. The last correctly finished task was counted as the maximum score.

Rivermead Motor Assessment Subtest arm is a subscale ("Upper Limb/Extremity" ('Arm')) of the Rivermead Motor Assessment that determines motor performance of patients after stroke, and consists of 15 arm movements such as pronating/supinating the forearm, bouncing a ball as well as functional items such as cutting putty, grasping and releasing objects, and tying a bow. It consists of test items in three sections that are ordered hierarchically, i.e., first items are easier and become more difficult towards the end of the evaluation. The subtest Arm is assessed as described above for the RMI.

Modified Ranking Scale (mRS):

Trained assessors received information of functional parameters from inpatient care centres and performed all ratings.

Actigraphy:

GT3x accelerometers were initialized using ActiLife Software, Version 6.8.2 (Actigraph Corp, Pensacola, USA) with 100 Hz sample rate and programmed to record the entire day (24 hrs. starting at 12 a.m.) starting the day after each study visit. The assessors explained the rationale of the devices and asked the patient to wear the device until the morning after the recording started. Patients were requested to wear the device the entire time and only take them off during washing or because of extreme discomfort. In order to guarantee a correct relocation of the device in case it was taken off, patients were shown how to place the device by the assessor. If patients suffered from severe paresis of the upper limb, personnel or relatives were instructed to handle the device. The actigraph was placed on the paretic ankle joint with the device pointing to the lateral side. For return shipping patients were provided with prepaid envelopes. Data were downloaded in 60-second intervals. Wear time validation as well as data scoring for step count assessment were executed using ActiLife software. As a cut-off point, we used more than 60 minutes of continuous zeros, with allowance of 1–2 minutes with counts between 1 and 100. Rated non-wear times were subsequently removed from the analyses. For step count, we applied the company-made low frequency extension filter to discriminate steps of slow walkers from random noise, as suggested by Webber & St. John.[3] As a cut-off we used the filter for patients exhibiting a walking speed ≤0.4 m/s.

Walking aids

During gait assessments (10m Walk West and 6-minute Walk Test) patients used the same walking aids and/or orthoses as during standard care physio therapy. All walking aids and orthoses were noted by trial assessors. For statistical analysis walking aids were dichotomised as follows.

0: no walking aid.

1: walking aid (walking frame, walking can, four point walking cane).

Nave, Rackoll et al.; p.11

Box and Block Test:

The Box and Block Test (BBT) (5) assesses gross manual dexterity of the upper limb to determine functional levels of the upper extremity in people with disability compared with those without disability. The test consists of two wooden boxes and a partition in the middle. One of the boxes is filled with 150 squared blocks with a length of the edges of 2.5 cm each. The patient is seated in front of the boxes. After a precise description of the task and verification that the task was understood correctly, the patient is asked to move as many blocks as possible over the partition. He or she is given 60 seconds per hand. After the 60 seconds the examiner counts the number of blocks the patient has moved. If the patient has moved two or more blocks at the same time those blocks are subtracted from the result.

Medical Research Council Scale:

The Medical Research Council (MRC) (6) scale is the accepted clinical tool for assessing muscle strength. It is rated on an ordinal scale ranging from 0 (plegic) to 5 (full strength), that has shown high intra- and inter-rater reliability. We evaluated the muscle force of six functional muscle groups (hip extension and flexion, knee extension and flexion as well as ankle extension and flexion) of the lower limb.

Trained study assessors compared the impaired with the non-impaired leg assessed the muscle strength. The force of the impaired leg is noted. Prior to the trial, all assessors validated their ratings against each other and against the trainer.

Resistance to passive movement scale:

The Resistance to passive movement scale (REPAS) (7) is a scale measuring spasticity in the lower and upper limbs, comprising eight muscle groups per side in the upper limb, five muscle groups per side in the lower limb, and one overall sum score. It is based on the Ashworth and the modified Ashworth scale, the most commonly used measures for spasticity/resistance to passive movement. The REPAS manual provides instructions for both test administration and scoring of various passive limb motions, showing high internal consistency and reliability for the clinical assessment of resistance to passive movement in patients with upper motor neuron paresis.

Within each muscle group spasticity is rated with a 4-point scale (0 denotes 'no increase in muscle tone', 4 denotes 'fixed in extension and flexion'). The patient is positioned on a bed, and asked to relax the assessed limb as much as possible. The assessor moves each limb starting slowly in the beginning. If neither spasticity nor pain is experienced the limb is moved more quickly to measure finer grades of spasticity.

Center for epidemiologic studies depression scale (CES-D):

The CES-D (8) is a 20-item measure in which patients rate how often they experienced symptoms associated with depression. Response options range from 0 to 3 for each item (0 = Rarely or None of the Time, 1 = Some or Little of the Time, 2 = Moderately or Much of the time, 3 = Most or Almost All the Time). Scores range from 0 to 60, with high scores indicating greater depressive symptoms. The questionnaire is handed to the patients and filled according to the official manual at each visit. Only in cases of severe paresis of the dominant hand or visual impairment the questionnaire is read to the patient and answers documented by the assessor.

The CES-D provides with a lie criteria which rates validity of the answers provided. We used -28 as a cut off and consequently excluded patients with a value below the cut off from analysis.

EuroQol quality of life questionnaire (EQ-5D-5L):

The EQ-5D-5L is a questionnaire in which the patients rate (1 = no problem – 5 = severe problem) their wellbeing within five dimensions as well as rate the impression of one's health on a vertical visual analogue scale. Questionnaires were filled by each patient at each visit except from those with paresis in their dominant hand. Calculations of EQ-5D-5L was done using the German validation set recently published.(9)

Pittsburgh Sleep Quality Index (PSQI):

The PSQI (10) is a measure to rate sleep and asks for reasons for bad sleep. It is filled at each visit. Except for baseline the PSQI asks for sleep behavior during the last four weeks preceding the visit. At baseline the PSQI is filled with respect to the sleep behavior within the four weeks prior to the cerebrovascular event.

Montreal Cognitive Assessment (MOCA):

The MOCA (11) is a screening tool for cognitive impairment and has a good validity in the stroke population and it provides with three different versions. It is filled at each visit. Except for the six months follow-up visit a different version is used at each assessment. At the six months follow-up visit the version from the baseline visit is filled.

Trail Making Test (TMT):

The trail making test (12) is a neuropsychological test which assesses executive functioning and task switching. The test is administered to each patient at each visit. Cases with an initial neglect symptom were excluded from analysis as the neglect was most likely to overshadow the performance in executive functioning.

Regensburger Wortflüssigkeitstest (RWT; word fluency):

The RWT asks for phonemic and semantic word fluency in four tasks. The sum score is equivalent to the words produced within each minute per tasks minus the mistakes. The test is only administered at baseline and at the three months follow-up visit.

Statistical Analysis Plan

A brief description of planned statistical analyses were published in the study protocol (14). The detailed statistical analysis plan (SAP) was published prior to unblinding (SAP published: Sept 1, 2017; last patient's follow-up visit: Nov 1, 2017) and can be found here:

https://doi.org/10.6084/m9.figshare.5375026.v1

Statistical Analysis Plan: Changes

Handling of missing values

- – In the analysis plan from the study protocol published in Trials (14) it was stated to use baseline value imputation in case of missing values.

In the SAP and in the final analyses, we used multiple imputation methods (MICE: multiple imputation by chained equations) for all missing data, except for data missing not at random (MNAR). This approach is regarded as the most appropriate method for missing value imputation in clinical trials (15).

Analyses of safety outcomes

- – In the SAP, it was stated to use Fisher's exact test when analysing safety outcomes.

In the primary analyses we used Poisson regression models instead to calculate incidence rates and incidence rate ratios. These models allow accounting for the time at risk for each patient, which differed in case of individual early termination of the trial.

Nave, Rackoll et al.; p.13

Statistical Analysis Plan: Imputation

Taking into account that our target population was moderately to severely affected, we expected missing values. Handling of missing values and definitions of missings are described here (15,16):

Table S3: Handling of missing data.

Missing data	Handling of missing data
Missing at random (MAR)	Occurrence was expected for follow-up timepoints. We imputed missing values with multivariate imputation by chained equations (17). Imputation was planned to be conducted using the 'miceadds' package of R Statistical Software. Dependent on the variable with missing values groupwise imputation was conducted using that variable with baseline and follow-up data, and additionally the variables centre, sex, age, baseline FAC, and treatment received. We imputed data on the basis of 10 datasets using the r-command: mice(dat_for_imp, m = 10, seed = 123) With default settings where 'dat_for_imp' denotes the selected variables. The seed was set at '123' to ensure data analysis reproducibility.
Missing completely at random (MCAR)	Occurrence was expected for logistic reasons or failure of measurement equipment. Thus no observed data was at hand to explain missings. Missings termed MCAR were treated in the same way as described for MAR.
Missing not at random (MNAR)	Occurrence was expected for patients unable to be assessed for the reason of general or specific impairment. For baseline we imputed missing values with single value imputation (18). If patients were not able to conduct an assessment due to their impairment as was seen in walking related assessments, single value imputation was used using half of the lowest value observed in the entire cohort. We decided to take the half of the lowest value of the group for patients that were not able to perform the desired task (MNAR), so that the mean of imputed values is lower than the mean of the rest of the cohort. This simple approach generates a very small individual maximal walking speed reflecting the moderately to severely impaired cohort without defining zero as a maximum walking speed at baseline, or loosing data points.

Data sharing

All scripts for the primary endpoint analyses as well as the corresponding datasets can be found here: http://doi.org/10.5281/zenodo.3341240

Supplementary Results

Protocol adherence

At one study site (Charité, Campus Benjamin Franklin) physiotherapists delivered all study interventions (PHYS and RELAX). In all other participating study centres, care providers were available on site delivering the respective study interventions (physiotherapists or sport therapists for PHYS; psychologists and neuropsychologists for RELAX).

Table S4: Additional baseline characteristics

Characteristic	PHYS-Group (n=105)	RELAX-Group (n=95)	All Patients (n=200)
Comorbidities			
Atrial Fibrillation, n (%)	23 (22)	23 (24)	46 (23)

Nave, Rackoll et al.; p.14

Diabetes mellitus, n (%)	32 (31)	31 (33)	63 (32)
Hypertension, n (%)	86 (82)	80 (84)	166 (83)
Hypercholesterolemia, n (%)	43 (41)	37 (39)	80 (40)
Coronary artery disease, n (%)	11 (11)	18 (19)	29 (15)
Tumor, n (%)	12 (11)	8 (8)	20 (10)
Medication			
Antiplatelets, n (%)	70 (67)	69 (73)	139 (70)
Oral anticoagulation, n (%)	20 (19)	20 (21)	40 (20)
Beta blocker, n (%)	49 (47)	47 (50)	96 (48)
Diuretics, n (%)	46 (44)	37 (39)	83 (42)
Calcium channel blockers, n (%)	41 (39)	38 (40)	79 (40)
Statins, n (%)	79 (75)	80 (84)	159 (80)
Antibiotics, n (%)	3 (3)	2 (2)	5 (3)
Analgetics, n (%)	13 (12)	18 (19)	31 (16)
Smoking‖			
Never smoked, n (%)	47 (53)	49 (57)	96 (55)
1-20 pack years, n (%)	19 (21)	14 (16)	33 (19)
>20 pack years, n (%)	23 (26)	23 (27)	46 (26)
Body Mass Index, mean (SD), kg/m^2	26 (4)	26 (4)	26 (4)

‖ History of smoking was not available in 25 patients.

Table S5: Parameters of protocol adherence per intervention group.

Intervention diaries	PHYS N = 105	RELAX N = 95
Time between baseline visit and intervention start in days, mean (SD)	4 (3)	4 (3)
Number of performed intervention sessions, mean (SD)	16 (6)	17 (5)
Time of attention by therapist per session, mean (SD)	44 (9)	43 (7)
Duration of core intervention in minutes, mean (SD)	21 (4)	24 (3)
Heart rate pre-session in bpm, mean (SD)	78 (10)	73 (10)

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Heart rate post-session in bpm, mean (SD)	94 (14)	71 (10)
Heart rate delta in bpm, mean (SD)	15 (9)	-2 (3)
Blood pressure pre-session in mmHg, mean (SD)	126/75 (11/9)	126/74 (12/8)
Blood pressure post-session in mmHg, mean (SD)	128/76 (12/9)	123/73 (12/8)
Visual analogue scale of perceived exertion, mean (SD)*	5 (0)	2 (0)
Reason for stopping intervention sessions§, mean (SD)		
Pain	0 (1)	0 (0)
Urge to urinate	0 (0)	0 (0)
Time	0 (0)	0 (0)
Fatigue	2 (4)	0 (0)
Refusal	0 (0)	0 (0)
Other	0 (1)	0 (0)

* Visual analogue scale from 0 to 10 where 0 stands for no perceived exertion and 10 for maximum perceived exertion. § Displayed are the mean (SD) number of times an intervention session was stopped for any of the listed reasons.

Table S6: Progression in training modalities over time.

	Day 1	Day 20
Distance in metres, mean (SD)	446 (332)	967 (540)
Speed in km/h, mean (SD)	1.6 (1.3)	2.4 (1.1)
Incline used on treadmill in %, mean (SD)	0.4 (1.0)	1.8 (2.1)

We performed a sensitivity analysis to compare adherence to PHYS intervention regimen in severely impaired patients (FAC 0 – 1) to the rest of the training cohort (FAC 2 – 5):

Tables S7: Sensitivity analysis comparing training responses between severely impaired patients to the rest.

	FAC 0 – 1 (n = 41)	FAC 2 – 5 (n = 63)
Sessions, mean (SD)	16 (7)	16 (6)
No. of stopping intervention due to fatigue, mean (SD)	3 (4)	2 (3)
Minutes per session, mean (SD)	21 (5)	21 (4)
Visual analogue scale, mean (SD)	6 (2)	5 (2)
Delta heart rate, mean (SD)	15 (9)	15 (9)

Protocol adherence: Target Heart Rate assessment

We compared the pragmatic PHYS-STROKE approach to estimate the desired target heart rate (THR) of 50 – 60% of a patient's maximum heart rate (maxHR) to a conventional approach from the American College of Sports Medicine (19) which was also used in previous stroke trials (20) calculating 55% of an estimated maxHR (see Table S7). The data presented in Table S8 demonstrate that the PHYS-STROKE approach resulted in a higher desired THR compared to the conventional approach aiming for 55% of an estimated maxHR. This difference was observed throughout all age groups, but was particularly pronounced in younger individuals. The mean age of patients of the PHYS group was 69 (12) years. Therefore, it can be assumed that sufficient cardiovascular stress was induced in patients of PHYS-STROKE allocated to aerobic physical fitness training.

Table S8: Comparison of two formulas to calculate a patient's target heart rate (50-60% of maximum heart rate).

Approach type, in bpm	40 years old	50 years old	60 years old	70 years old	80 years old	90 years old
PHYS-STROKE approach (THR= 180-age)	140	130	120	110	100	90
Conventional approach (55% of maxHR)	99	95	91	87	83	79

The pragmatic PHYS-STROKE approach calculated the target heart rate (THR) with the formula: $THR = 180 - age$. The conventional approach calculated 55% of an estimated maximum heart rate (maxHR) with the formula: $THR = 0.55*(207 - 0.7 * age)$.

Outcomes: Intervention facilities

Four out of seven study sites had a gait trainer available. We performed a sensitivity analyses on severely affected patients (FAC < 3) who were not trained on a gait trainer as described in the protocol but no difference to the primary outcomes were observed.

Outcomes: Imputation

Data needed to be imputed for missing values due to attrition or calculated for patients too severely affected to complete the assessment of gait. Data imputation displayed is for co-primary and key secondary outcomes.

Table S9: Imputation of missing data.

Reason	All	PHYS	RELAX
Multiple imputation for gait speed of patients not available at follow-up visit 3 months post stroke, no (%). Assuming missing at random MAR.	34 (17%)	16 (15%)	18 (19%)
Calculated data for gait speed of patients not able to walk ten metres at baseline by using half of the lowest value of the cohort, no (%). Assuming missing not at random MNAR.	21 (11%)	9 (9%)	12 (13%)
Multiple imputation for Barthel-Index due to missing values of patients at follow-up visit 3 months post stroke, no (%)∫. Assuming MAR.	32 (16%)	15 (14%)	17 (18%)

∫Two patients (1 PHYS, 1 RELAX) were not present at follow-up visit 3 months post stroke, but the assessment of the Barthel-Index was possible via telephone.

We performed a sensitivity analysis to assess the quality of our multiple imputation approach by giving the imputation a hierarchical order as this has been suggested previously (21). We used centre as the highest order, to impute missing data in a groupwise fashion. However, no improved assumption to missing data was found using hierarchical order as outlined in Table S9. Although it seems reasonable to cluster data in a hierarchical order, in our case at least, centre did not improve the outcome, most likely because of the low number of data in each centre.

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Table S10: Sensitivity analysis of co-primary endpoints with hierarchical imputation.

Variable	PHYS (n=105)	RELAX (n=95)	Treatment effect / OR (95% CI)	P Value
Co-Primary Outcomes				
Change in maximal walking speed, mean (95% CI), in m/s	0.4 (0.2 to 0.6)	0.3 (0.0 to 0.6)	0.1 (-0.1 to 0.3)	0.46
Change in Barthel-Index, mean (95% CI)	32 (25 to 39)	30 (19 to 41)	0 (-12 to 12)	0.97

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Outcomes: Exploratory analysis of subgroups
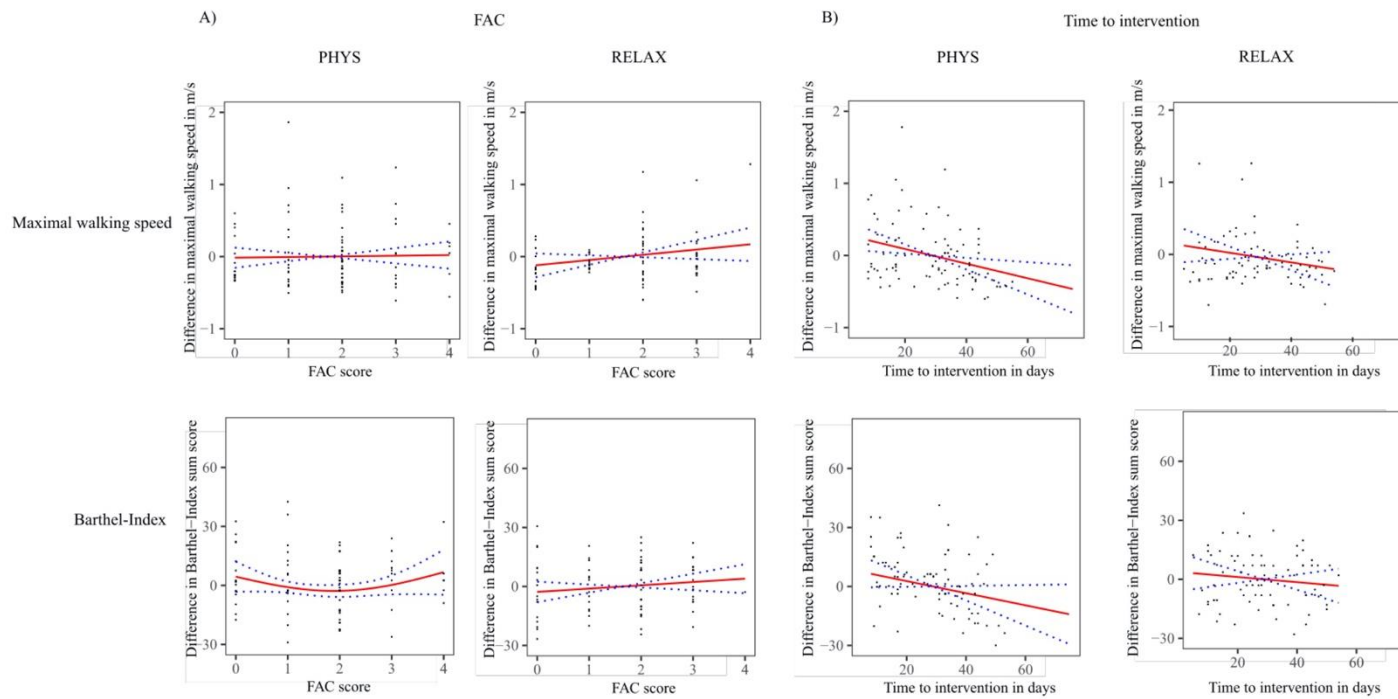
Figure S2: Subgroup analyses demonstrating the difference in maximal walking speed and Barthel-Index (follow up three months after stroke - baseline) as a function of Functional Ambulation Category (FAC, panel A) and the time from stroke onset to start of intervention (Time to intervention, panel B), respectively, using splines.

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Standard care

Times of standard care therapies depend on the degree of functional disability. Data have thus a skewed distribution.

Table S11: Inpatient and outpatient therapy times between baseline and follow-up 3 months post stroke and during intervention period only.

Observation time: Baseline - 3 months post stroke	PHYS n = 105	RELAX n = 95	ALL n = 200
Physiotherapy, median [IQR], min¶	2220 [1545 – 2782]	2122 [1540 – 2692]	2160 [1530 – 2760]
Occupational therapy, median [IQR], min	1860 [1155 – 2355]	1560 [1140 – 2145]	1680 [1140 – 2284]
Speech therapy, median [IQR], min	960 [600 – 1335]	915 [570 – 1331]	930 [570 – 1335]
Neuropsychological therapy, median [IQR], min	690 [428 – 1008]	600 [480 – 840]	670 [450 – 900]
Observation time: Intervention period			
Physiotherapy, median [IQR], min¶	1320 [945 – 1680]	1230 [840 – 1733]	1268 [895 – 1710]
Occupational therapy, median [IQR], min	1050 [600 – 1350]	960 [583 – 1350]	1043 [599 – 1350]
Speech therapy, median [IQR], min	450 [210 – 750]	420 [188 – 655]	450 [210 – 825]
Neuropsychological therapy, median [IQR], min	380 [225 – 510]	360 [210 – 450]	360 [210 – 480]

Data comprise the total of applied inpatient and outpatient therapies in minutes from baseline until follow-up visit three months after stroke. Data of 55 patients were not provided by the study sites.

¶Physical therapy comprises either conventional Bobath therapy or repetitive locomotion therapy depending on the trial site's standard care protocols. Statistical analyses are adjusted for study sites.

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Supplementary information for patients and carers

Definitions for acute, subacute and chronic phases are defined as follows and are in line with definitions endorsed by the Stroke Recovery and Rehabilitation Roundtable (SRRR) (22):
Hyperacute: <24 hours post stroke
Acute: 1 – 7 days post stroke
Early Subacute: 1 week – 3 months post stroke
Late Subacute: 3 months – 6 months post stroke
Chronic: > 6 months post stroke

Physical exercise recommendations after stroke:
https://www.ahajournals.org/doi/full/10.1161/STR.0000000000000022

Rehabilitation recommendations:
https://www.nice.org.uk/guidance/cg162/resources/cg162-stroke-rehabilitation-full-guideline3

Standard care physiotherapy after stroke:

https://www.stroke.org.uk/resources/physiotherapy-after-stroke

Barthel-Index:
http://www.strokecenter.org/wp-content/uploads/2011/08/barthel.pdf

Bobath approach:
https://www.physio-pedia.com/Bobath_Approach (English)
https://de.wikipedia.org/wiki/Bobath-Konzept (German)

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11.   Publikation 2: Physical Exercise in Patients with Subacute Stroke (PHYS-STROKE): safety analyses of a randomized clinical trial

Shared first authorship: Rackoll & Nave

Journal Data Filtered By: **Selected JCR Year: 2019** Selected Editions: SCIE,SSCI
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21	Neurotherapeutics	4,998	6.035	0.009520
22	Translational Stroke Research	2,274	5.780	0.004520
23	BRAIN PATHOLOGY	5,308	5.568	0.007020
24	Pain and Therapy	354	5.526	0.001230
25	PAIN	37,753	5.483	0.035730
26	Multiple Sclerosis Journal	11,792	5.412	0.019460
27	BIPOLAR DISORDERS	4,838	5.410	0.006610
28	Therapeutic Advances in Neurological Disorders	1,421	5.000	0.002960
29	International Journal of Stroke	4,853	4.882	0.015560

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Physical Fitness Training in Patients with Subacute Stroke (PHYS-STROKE): Safety analyses of a randomized clinical trial

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Abstract

Background and aim: To report the six-month safety analyses among patients enrolled in the "Physical Fitness Training in Subacute Stroke—PHYS-STROKE" trial and identify underlying risk factors associated with serious adverse events.

Methods: We performed a pre-specified safety analysis of a multicenter, randomized controlled, endpoint-blinded trial comprising 200 patients with moderate to severe subacute stroke (days 5–45 after stroke) that were randomly assigned (1:1) to receive either aerobic, bodyweight supported, treadmill-based training (n = 105), or relaxation sessions (n = 95, control group). Each intervention session lasted for 25 min, five times weekly for four weeks, in addition to standard rehabilitation therapy. Serious adverse events defined as cerebro- and cardiovascular events, readmission to hospital, and death were assessed during six months of follow-up. Incident rate ratios (IRR) were calculated, and Poisson regression analyses were conducted to identify risk factors for serious adverse events and to test the association with aerobic training.

Results: Six months after stroke, 50 serious adverse events occurred in the trial with a higher incidence rate (per 100 patient-months) in the training group compared to the relaxation group (6.31 vs. 3.22; IRR 1.70, 95% CI 0.96 to 3.12). The association of aerobic training with serious adverse events incidence rates were modified by diabetes mellitus (IRR for interaction: 7.10, 95% CI 1.56 to 51.24) and by atrial fibrillation (IRR for interaction: 4.37, 95% CI 0.97 to 31.81).

Conclusions: Safety analysis of the PHYS-STROKE trial found a higher rate of serious adverse events in patients randomized to aerobic training compared to control within six months after stroke. Exploratory analyses found an association between serious adverse events occurrence in the aerobic training group with pre-existing diabetes mellitus and atrial fibrillation which should be further investigated in future trials.

Data access statement: The raw data and analyses scripts are provided by the authors on a secure online repository for reproduction of reported findings.

Keywords
Aerobic treadmill training, subacute stroke, stroke rehabilitation, serious adverse events, safety

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Introduction

The number of stroke survivors with impairments is increasing, rendering effective rehabilitation interventions a major unmet medical need.[1] Aerobic training is a recommended treatment modality in stroke rehabilitation to counter cardiorespiratory deterioration.[2–4] However, it remains uncertain whether training in the critical early period of stroke recovery can be carried out safely. Cardiorespiratory stress applied during early rehabilitation might cause adverse effects.[5]

The evidence of safety of aerobic training early after stroke is scarce. The latest Cochrane Collaboration meta-analysis aggregated estimates of adverse effects including cerebro- and cardiovascular events in the stroke population but could not identify a higher risk in aerobic training compared to control interventions.[6] Of note, the evidence derived mainly from small studies with limited reporting of adverse events.

Surprisingly and in contrast to smaller stroke rehabilitation trials, the results of the recent "Physical Fitness Training in Subacute Stroke" (PHYS-STROKE) trial,[7] which randomized subacute stroke patients to early aerobic training or relaxation, identified a higher risk of serious adverse events (SAE) within three months post stroke in the training group compared to control.

Aims

In accordance with the trial protocol, we now report the final safety data of the six-month trial follow-up and additionally provide exploratory analyses aimed to identify patient-related risk factors for SAE associated with aerobic training. The six months' follow-up was chosen to unveil longer term effects of an early aerobic training in the subacute phase after stroke.

Methods

The trial protocol,[8] statistical analysis plan (https://doi.org/10.6084/m9.figshare.5375026.v1), and the primary efficacy endpoints of the multi-center, randomized, controlled PHYS-STROKE trial (clinicaltrials.gov identifier: NCT01953549) were published previously.[7] The protocol was approved by the local ethics committee of the Charité Universitätsmedizin Berlin (EA1/138/13). Patients with either ischemic or hemorrhagic stroke in the subacute phase (5–45 days post onset) were included into the trial. The inclusion and exclusion criteria of the trial are listed in the Supplementary Table 1. All patients gave written informed consent.

Detailed intervention procedures have been previously described.[7] In brief, patients were randomly assigned to receive either bodyweight supported, treadmill-based, aerobic training or relaxation sessions, in addition to standard rehabilitation therapy over a period of four weeks with five sessions per week á 25 min each. The target heart rate (THR) in the training group was calculated by the formula: 180 – "years of age", which was hypothesized to approximate 50–60% of each patient's maximum heart rate. The THR was reduced by 10 beats per minute in case of β-blocker medication.[9]

Pre-defined safety endpoints included the following SAE: recurrent non-fatal cardio- or cerebrovascular event, readmission to an acute care hospital, or death within six months post stroke. Cerebrovascular event included any stroke or transient ischemic attack confirmed by cerebral imaging with or without clinical manifestation that led to new ICD-10 diagnosis. Readmission to an acute care hospital had to be confirmed by discharge letter.

All SAE occurring during hospitalization at the rehabilitation clinic were monitored by a study site physician and had to be reported within 24 h after onset to the coordinating trial center. SAE that occurred after discharge from hospital were inquired from the trial participants or their relatives and confirmed by discharge letters during clinical follow-up visits at three and six months post stroke. Access to the population registry of Berlin was requested to retrieve current health status for patients unavailable at the six-month follow-up. Medical monitoring appointed by the Center for Stroke Research Berlin checked data fidelity and reporting of SAE at each study site. An independent data and safety monitoring board (DSMB) overlooked all SAE on a regular basis.

As part of the accompanying biomarker study "Biomarkers And Perfusion – Training-Induced Changes After Stroke" (BAPTISe), cerebral magnetic resonance imaging (MRI) was acquired for a subsample of patients (n = 110) before and after the study intervention.[10] New ischemic lesions visible on diffusion-weighted imaging with or without clinical manifestation were reported to the coordinating trial center. In an exploratory framework, we analyzed relationships of SAE occurrence with patient characteristics, pre-existing comorbidities, medication, and pertinent blood biomarkers (details in supplementary appendix). All comorbidities needed to have a formal diagnosis issued with the respective ICD code at baseline.

Descriptive summary statistics are presented as mean and standard deviation (SD) or median and interquartile range (IQR) where appropriate. The pre-defined safety endpoints were analyzed using Poisson regression models with individual observation time until six months of follow-up as time at risk, which allows for calculating incidence rates (IR) and incidence

rate ratios (IRR) with 95% confidence intervals (CI).[11] We calculated mixed Poisson regression models with number of SAE as a dependent variable, treatment arm as an independent variable, and a random effect (random intercept) to account for center heterogeneity. Additionally, we incorporated individual observation times (log-transformed time of observation as offset). Sensitivity analysis was performed without recurrent strokes detected on cerebral MRI within the BAPTISe study.

For our exploratory risk factor analysis, we fitted multiple Poisson regression models to assess the association of preexisting comorbidities, treatment group, and SAE. Models were controlled for age, sex, and stroke severity to test robustness of associations. Well-established cardiovascular risk factors, i.e. arterial hypertension, atrial fibrillation, diabetes mellitus, history of cerebrovascular event, history of cardiac disease, number of comorbidities, and related medication (antiplatelets, oral anticoagulants, β-blocker, and statins) were added to the models. Additionally, two-way interaction terms with respective risk factors and treatment group were tested. Decision on the final models was based on Bayesian information criterion (BIC). This was done to achieve parsimonious models with high exploratory power, to avoid overfitting and to avoid inclusion of highly correlated variables. Additionally, to illustrate the direction of interaction effects, we used estimated marginal means of subgroups from the final model. Kaplan-Meier curves were used to verify found associations and to illustrate time to first SAE in relevant risk factor subgroups. All analyses are exploratory with regard to the original analysis plan and were not corrected for multiple testing (see Supplements).

Results

The PHYS-STROKE trial randomized 200 patients (training, n = 105 *vs.* relaxation, n = 95) of which 190 (95%) were followed-up until six months post stroke (Figure 1). Four patients of the training group (4%) and six patients of the relaxation group (6%) were lost to follow-up. Eight patients (training, n = 5 vs. relaxation, n = 3) had to discontinue the intervention due to SAE. Protocol adherence and baseline characteristics can be found in Supplementary Tables 2 and 3. Patients had a mean age of 69 years (SD 12 years) and 41% were females. Incident strokes were predominantly ischemic (90%) with a median acute National Institute of Health Stroke Scale (NIHSS) score of 8 (5 to 12). Patients randomly allocated to the training group were more severely affected than patients in the relaxation group (acute NIHSS: 9 (5–12) vs. 7 (5–11)).

Over the course of the trial, 50 SAE occurred in 39 patients. Fifteen recurrent cerebrovascular events and

30 readmissions to an acute care unit were observed. Five patients died within the observation period (training, n = 1 vs. relaxation, n = 4). All fatal events took place after the intervention phase. No cardiovascular event was recorded. Median time from start of intervention to SAE occurrence was 41 days (IQR 14–111). Eighteen SAE (36%) appeared during the intervention phase (training: eight strokes and six readmissions; relaxation: four readmissions) but none during an intervention session. Nine patients (training, n = 6 vs. relaxation, n = 3) had more than one SAE. Individual SAE are listed in the Supplementary Table 4.

Recurrent cerebrovascular events were due to ischemic stroke (n = 13, 10 in the training and 3 in the relaxation group) or transient ischemic attacks (n = 2, one per group). Five ischemic strokes (training, n = 4 vs. relaxation, n = 1) were asymptomatic incidental findings on baseline (n = 2) or post-intervention (n = 3) MRI within the BAPTISe study. Hospital readmissions primarily occurred because of cardiac complications (27%) and are listed in the Supplementary Table 5. In the training group, one patient died due to an urosepsis, and in the relaxation group, two patients died from a recurrent cerebral infarction, one from an acute aortic dissection, and one cause remained unknown. All fatal events were judged unrelated to the intervention by the DSMB. Three recurrent ischemic events in the training group were judged as "possibly although unlikely related to the intervention"; all other SAE were judged "unlikely" to be related to the intervention.

Incidence rates for SAE from Poisson regression analyses are presented in Table 1. Comparing aerobic training with relaxation, within six months of follow-up, the risk per 100 patient-months was 2.3 events and 0.9 events for recurrent cerebrovascular events (IRR: 2.43, 95% CI 0.83 to 8.76, p = 0.13), 4.3 and 2.1 for readmission to an acute hospital (IRR 2.06, 95% CI 0.97 to 4.73, p = 0.07), and 0.11 and 0.93 for fatal events (IRR of 0.22; 95% CI 0.01 to 1.50, p = 0.18), respectively. Sensitivity analysis after exclusion of any incidental MRI findings from the "BAPTISe" substudy (n = 5) demonstrated similar IRR for cerebrovascular events (IRR 2.06, 95% CI 0.58 to 9.59).

Distributions of patient characteristics with SAE occurrence are displayed in the Supplementary Table 6. Unadjusted Poisson regression models identified a higher risk of SAE in patients with vs. patients without arterial hypertension in the training group compared to the relaxation group. Similarly, this was observed for patients with diabetes mellitus (DM), atrial fibrillation (AF), higher hs-CRP, and higher serum cortisol.

We fitted multiple Poisson regression models with respective preexisting comorbidities and cardiovascular risk constellations and identified parsimonious models

Figure 1. Flow chart of patient enrollment and follow-up of the PHYS-STROKE trial.
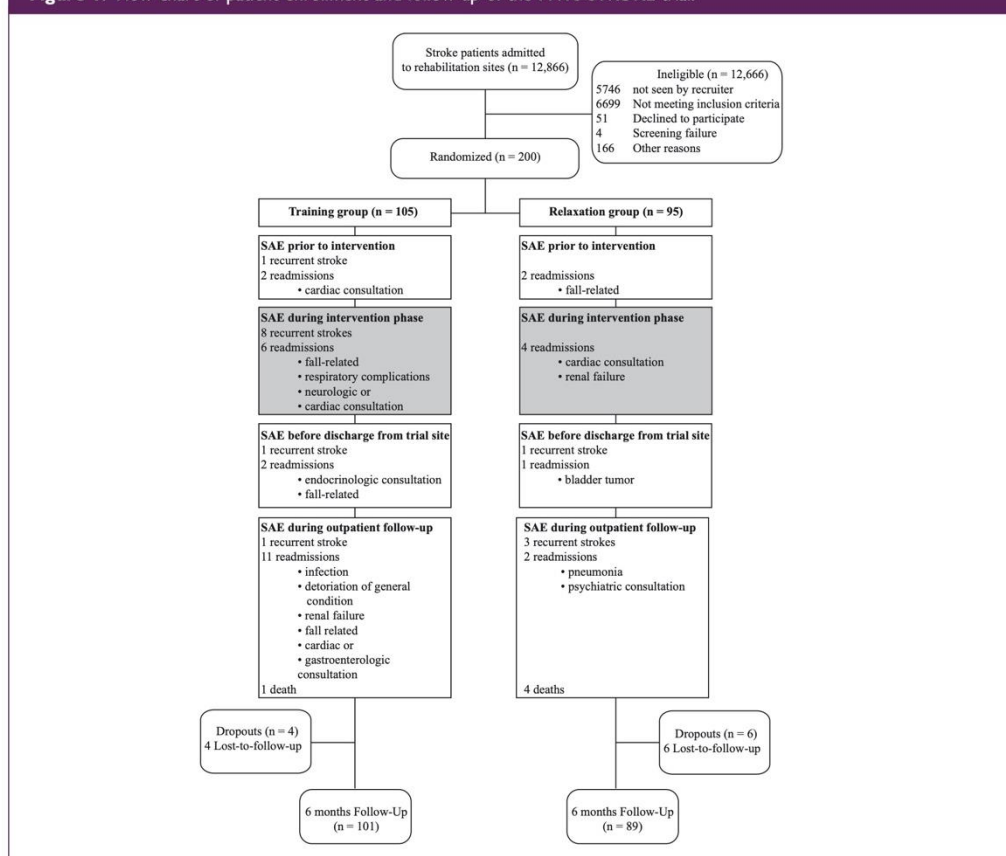
with best explanatory power (identified by BIC) analyzing the association of DM and AF with SAE with an interaction between treatment and DM (IRR for interaction 7.10, 95% CI 1.56 to 51.24; Figure 2(a)) and between treatment and AF (IRR for interaction 4.37, 95% CI 0.94 to 31.81; Figure 2(b)), respectively, after adjusting for age, sex, and NIHSS. Kaplan-Meier curves illustrate the course of SAE occurrence for DM and AF in Figure 3. Details of models and model selection can be found in Supplementary Table 7. Due to the small number of SAE and due to collinearities, it was not possible to derive one model including the association of DM and AF with SAE in parallel.

Discussion

The results of this analysis from a randomized, controlled stroke rehabilitation trial extends the current knowledge of safety of an early bodyweight supported, treadmill-based aerobic training after stroke and provides evidence of potential harms compared to a relaxation program in the early subacute phase after moderate to severe stroke. We detected 50 SAE within six months after stroke which corresponds to a higher incidence rate of SAE than has been reported in previous trials.[6] Additionally, exploratory analyses identified potential risk factors for SAE. When randomized to the aerobic training group, patients with pre-existing diabetes mellitus or atrial fibrillation had a seven-fold or four-fold higher risk of experiencing SAE, respectively. In contrast, risks for SAE were similar for patients without DM or without AF in both intervention groups.

So far, aerobic training was deemed safe in the stroke population.[6] In our trial, SAE occurred more frequently in the aerobic training group with a peak

Table 1. Serious adverse events until six months post-stroke

	All N = 200	Training N = 105	Relaxation N = 95	IRR (Incidence-Rate-Ratio) (95% CI)	p
Follow-up time (in days), median [IQR]	153 [139–164]	154 [140–166]	153 [135–163]		
Total SAE IR 95% CI	50 4.60 (1.99–8.53)	33 6.31 (2.89–10.82)	17 3.22 (0.87–6.91)	1.70 (0.96–3.12)	0.07
Cerebrovascular event IR 95% CI	15 1.58 (0.59–2.86)	11 2.25 (1.04–3.88)	4 0.93 (0.29–2.15)	2.43 (0.83–8.76)	0.13
Cardiovascular event	0	0	0	–	–
Readmission to hospital IR 95% CI	30 3.07 (1.45–5.08)	21 4.30 (2.23–6.69)	9 2.08 (1.00–3.75)	2.06 (0.97–4.73)	0.07
Death IR 95% CI	5 0.54 (0.09–1.17)	1 0.11 (0.00–0.89)	4 0.93 (0.19–2.15)	0.22 (0.01–1.50)	0.18

Note: Incidence rates (per 100 patient-months) and incidence rate ratios of serious adverse events between both intervention groups.

Figure 2. Model-based estimates of events in subgroups of SAE for status of DM (a) and AF (b) in both treatment groups per 100 patient-months. Estimated marginal means are calculated from Poisson regression with Treatment, respective comorbidity, β-blocker medication and an interaction term for treatment with respective comorbidity and are adjusted for age, sex and NIHSS. Results are shown with 95% CI.
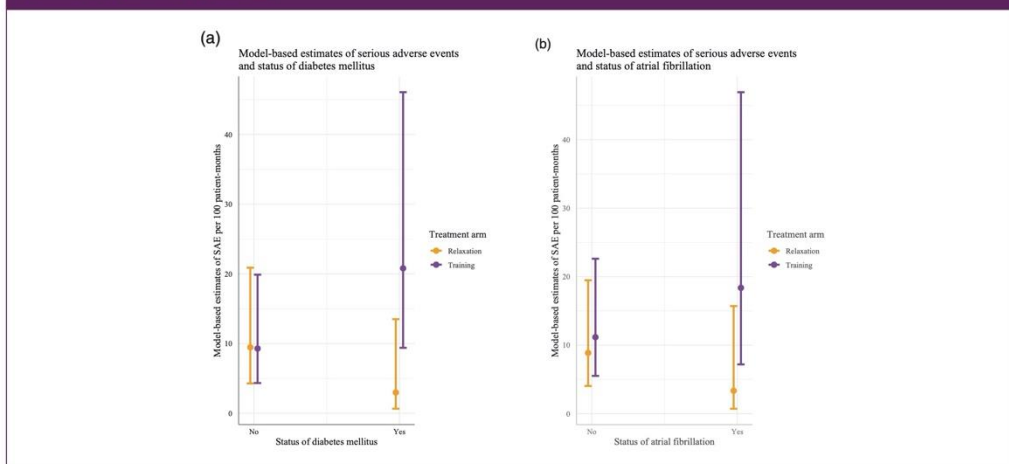
during the intervention phase. Particularly, recurrent cerebrovascular events happened in temporal relation with the training intervention, whereas patients in the relaxation group had no similar event.

Case fatality rates were lower in the training group after six months of follow-up compared to the relaxation group.

A detailed risk assessment of aerobic training is urgently needed, given that guidance for carers of

Figure 3. Kaplan–Meier curve of cumulated SAE occurrence stratified by DM (a) and AF (b) status over time. Interactions are shown as treatment with or without comorbidity.
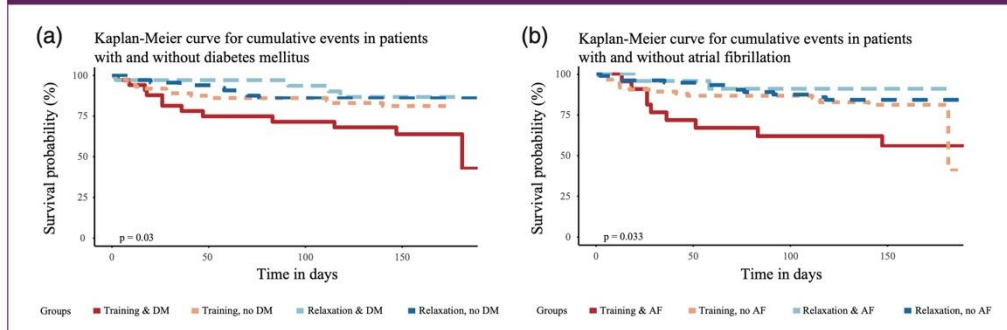
stroke patients remains contradictory and does not systematically account for individual patient characteristics.[2–4,12,13] So far, adverse events are rarely reported in stroke rehabilitation trials investigating aerobic training, and associations of adverse events with comorbidities are not discussed.[6] In fact, only 4 out of 17 trials on aerobic, treadmill-based exercise in the latest Cochrane review reported preexisting comorbidities of study participants in their publications, and in only seven publications, SAE assessment was described.[6] None of these reports tested for an association of specific patient characteristics and SAE occurrence. The PHYS-STROKE trial, however, reported a possible relation of patient characteristics to SAE occurrence for the first time and aimed to identify subgroups of patients with higher risk.

Diabetic patients are at higher risk for recurrent vascular events and poor outcome after stroke compared to patients without diabetes.[14] We found a higher association of DM with aerobic exercise in our study population. To the best of our knowledge, no literature exists investigating the risk of aerobic training in stroke patients with DM.

Presence of AF was associated with a higher risk of SAE occurrence in patients undergoing aerobic exercise in the present study. In general, patients with AF demonstrate a high risk of stroke.[15] Literature on tolerability of aerobic training in stroke patients with AF is scarce. So far, exercise is recommended for all patients with AF, but large exercise intervention studies with assessment on safety are still missing.[16] In a meta-analysis of exercise rehabilitation trials comprising patients with AF but without stroke, aerobic exercise was considered safe.[17] The American Association of Cardiovascular and Pulmonary Rehabilitation recommends to slowly progress in exercise intensity in early cardiac rehabilitation.[18] This may be also applicable in

early stroke rehabilitation for patients with concomitant AF, a hypothesis to be explored systematically in future trials.

The PHYS-STROKE trial entails the largest number of subacute stroke patients receiving aerobic training within a randomized clinical trial so far, but several limitations should be considered when interpreting our findings: First, the PHYS-STROKE trial was not powered to detect SAE. However, our trial was the first large study to conduct aerobic training in moderate to severe subacute stroke patients. Further research with rigorous reporting on adverse events is necessary. Second, despite randomization, stroke severity was not balanced between both treatment arms. Patients in the training group had more severe strokes as indicated by the NIHSS, which might have put patients at higher risk for SAE. However, NIHSS score was not substantially associated with SAE occurrence after adjustment for other covariates. Third, we used a pragmatic approach in our trial to assess THR of patients which may have over- or under-estimated the THR that would have been elicited by gradual exercise testing. Therefore, associations between intensity of aerobic training and rate of adverse events should be interpreted with caution. Fourth, the exact mechanisms underlying SAE occurrence following aerobic training cannot be determined in our study. Tentatively, SAE occurrence might also be related to preexisting comorbidities (see Supplementary Tables 3 and 4). However, comorbidities were similarly high in both intervention groups. Fifth, information on risk factors associated with cardiovascular events such as level of previous physical activity or diet were not assessed in the PHYS-STROKE trial. Although data on pre-stroke behavior might be vital, assessment of such measures are subject to recall bias. Lastly, our trial incorporated a rigorous training regimen with training sessions five

times per week over a four-week period. A slower progression of training intensity might be necessary to reduce potential risks in a vulnerable patient population.

Aerobic fitness training early after moderate to severe stroke may cause harm compared to relaxation, and increased risk of SAE was particularly attributed to patients with preexisting diabetes mellitus or atrial fibrillation in the PHYS-STROKE trial. Future trials are needed to confirm or refute these exploratory findings.

Data Availability Policy

The raw data and analyses scripts are provided by the authors on a secure online repository for reproduction of reported findings (https://doi.org/10.5281/zenodo.3899830).

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: All authors declare no financial relationships with any organization that might have an interest in the submitted work. TR reports no disclosure. AHN is participant in the Berlin Institute of Health BIH-Charité clinical scientist programme and reports grants from BIH during the conduct of the study. ME reports no disclosure. MEn reports grant support from Bayer, the German Research Foundation under Germany's Excellence Strategy—EXC-2049—390688087, the German Federal Ministry of Education and Research, the German Center for Neurodegenerative Diseases, the German Centre for Cardiovascular Research, the European Union, Corona Foundation, and Fondation Leducq; fees paid to the Charité from Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Pfizer, Daiichi Sankyo, Amgen, GlaxoSmithKline, Sanofi, Covidien, Novartis, all outside the submitted work. UG reports financial support of Centogene AG, outside of the submitted work. AF reports grant support from the German Research Foundation, German Federal Ministry of Education and Research, the German Center for Neurodegenerative Diseases, European Union, Else Kröner Fresenius Stiftung, and Hannelore Kohl Stiftung; consultant fees from Novartis and Bayer, and honoriums for presentations in scientific symposia by Novartis and Bayer, all outside the submitted work.

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Supplemental material

Supplemental material for this article is available online.

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Supplementary Appendix

Physical Exercise in Patients with Subacute Stroke (PHYS-STROKE): safety analyses of a randomized clinical trial

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Table of contents

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Authors and study group

Authors

Name	Location	Contribution
Torsten Rackoll, Dipl	Berlin Institute of Health, Berlin	Acquired, analyzed, and interpreted the data; conceptualized and designed the study; performed statistical analysis; drafted the manuscript for intellectual content
Alexander H. Nave, MD	Charité Universitaetsmedizin Berlin	Conceptualized and designed the study; acquired and analyzed the data; drafted the manuscript for intellectual content
Martin Ebinger, MD	Medical Park Humboldtmühle, Berlin	Conceptualized and designed the study; interpreted the data; revised the manuscript for intellectual content
Matthias Endres, MD	Charité Universitaetsmedizin Berlin	Interpreted the data; revised the manuscript for intellectual content
Ulrike Grittner, PhD	Charité Universitaetsmedizin Berlin	Conceptualized and designed the study; supervised the statistical analysis and interpretation of data; revised the manuscript for intellectual content
Agnes Flöel, MD	University Medicine Greifswald, Greifswald	Conceptualized and designed the study; supervised the study; major role in design of the PHYS-Stroke trial; revised the manuscript for intellectual content

Co-Investigators of the PHYS-Stroke study group

Name	Location	Role	Contribution
Andreas Meisel, MD	Charité Universitaetsmedizin Berlin	Chief Executive Director, Center of Stroke Research Berlin	Design and conceptualization of the PHYS-Stroke trial; Fundraising
Stefan Hesse, MD (deceased)	Charité Universitaetsmedizin Berlin	Chair	Design and conceptualization of the PHYS-Stroke trial
Heinrich J. Audebert, MD	Charité Universitaetsmedizin Berlin	Site investigator	Design and conceptualization of the PHYS-Stroke trial
Fabian Klostermann, MD	Charité Universitaetsmedizin Berlin	Site investigator	Site coordination and recruiting
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Darius G. Nabavi	Vivantes Klinikum Neukölln	Site investigator	Site coordination and recruiting

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Supplementary Methods

Table 1: Inclusion and exclusion criteria

	INCLUSION CRITERIA
1.	Diagnosis of stroke (inclusion within 5-45 days after stroke onset); ischemic or haemorrhagic (cortical, subcortical, brainstem), as determined by initial MRI/CT scan of the brain)
2.	Age ≥ 18 years
3.	Able to sit unsupported (i.e. without holding onto supports such as the edge of the bed), with feet supported, for at least 30 seconds
4.	Barthel-Index ≤ 65 at inclusion
5.	Considered able to perform aerobic exercise, as determined by responsible physician
6.	Provision of written informed consent
	EXCLUSION CRITERIA
1.	Patient considered unable to comply with study requirements
2.	Stroke due to intracranial haemorrhage primarily due to bleeding from ruptured aneurysm or arteriovenous malformation
3.	Progressive stroke
4.	Unable to perform the required exercises due to a) medical, b) musculo-skeletal, or c) neurological problems (for details see below, 4a-c)
4a.	Medical problems: unstable cardiovascular condition, or other serious cardiac conditions (e. g., New York Heart Association criteria for Class IV heart disease, hospitalization for myocardial infarction or heart surgery within 120 days, severe cardiomyopathy or documented serious and unstable cardiac arrhythmias)
4b.	Musculoskeletal problems: restricted passive range of motion in the major lower limb joints (i.e. an extension deficit of > 20° for the affected hip or knee joints, or a dorsiflexion deficit of > 20° for the affected ankle)
4c.	Neurological problems: severity of stroke-related deficits
5.	Required help of at least 1 person to walk before stroke due to neurological (e. g., advanced Parkinson's disease, Amyotrophic Lateral Sclerosis, Multiple Sclerosis) or non-neurological co-morbidities (e. g. heart failure, orthopaedic problems)
6.	Life expectancy of less than 1 year as determined by responsible physician
7.	Drug or alcohol addiction within the last six months
8.	Significant current psychiatric illness defined as medication-refractory of bipolar affective disorder, psychosis, schizophrenia or suicidality.
9.	Current participation in another interventional trial

MRI = magnetic resonance imaging | CT = computer tomography

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Control intervention

The active control group received relaxation sessions for the same duration as the training group. Relaxation focused on contraction and relaxation of muscle groups of the upper body. In both intervention groups, the heart rate of patients was monitored throughout each session.

Statistics

As exploratory secondary safety analyses, we analyzed the influence of SAE on previously reported primary efficacy endpoints, hospitalization time and discharge to aftercare facility. Additional exploratory analyses were performed to explore the influence of SAE occurrence on previously reported efficacy endpoints. Linear mixed effects regression was used to calculate the difference in hospitalization time and multinomial regression was used to estimate odds ratios (OR) for discharge to aftercare. In post-hoc analyses, blood biomarkers were used to test if associated comorbidities in risk factor analyses were adequately controlled. Statistical tests presented in the main manuscript as well as in the supplemental material were done in R statistical software version 3.6.2 with the 'tableone'[14] and the 'lme4'[15] package.

Blood biomarker analysis

The following blood-derived biomarkers were analyzed using standard procedures and are included as part of participants' baseline characterization in the supplements: Hemoglobin, hematocrit, erythrocytes, leukocytes, thrombocytes, glucose, insulin, HbA1c, high density lipoprotein, low density lipoprotein, triglycerides, lipoprotein a, kidney, creatinine, estimated glomerular filtration rate (eGFR), fibrinogen, high-sensitivity C-reactive protein (hs-CRP), tumor necrosis factor alpha (TNF-alpha), interleukin 6 (IL-6), cortisol, and thyroid-stimulating hormone (TSH).

Fibronogen:

The quantitative determination of fibrinogen levels was performed based on the Clauss method in citrated blood plasma by using the HemosIL® Q.F.A. Thrombin (Bovine) kit, Instrumentation Laboratories.

CRP, TnF-a, IL-6:

Blood levels of high-sensitive C-reactive protein (hs-CRP), tumor necrosis factor-alpha (TNF α), and interleukin-6 (IL-6) were quantitatively determined using solid-phase, chemiluminescent immunometric assays (IMMULITE® 1000, Siemens Healthcare Diagnostics).

Insulin:

Serum insulin levels were quantified with a electrochemiluminescence immunoassay "ECLIA" using cobas® Elecsys immunoassay systems, Roche Diagnostics.

Lp(a):

Lipoprotein(a) was quantified by kinetic nephelometry (Immage Immunchemie System, Beckmann Coulter) using a polyclonal antibody in an assay insensitive to apo(a) isoforms.

Data Availability Policy

The raw data and analyses scripts are provided by the authors on a secure online repository for reproduction of reported findings (https://doi.org/10.5281/zenodo.3899830). Data include anonymized individual patient variables for results reported here, and analyses scripts used in this study. Data will be available with the publication of the article. Further information can be shared on individual request addressed to the corresponding author.

Supplementary Results

Table 2: Protocol adherence

Intervention	Training N = 105	relaxation N = 95
Number of performed intervention sessions, mean (SD)	16 (6)	17 (5)
Duration of core intervention in minutes, mean (SD)	21 (4)	24 (3)
Heart rate delta in bpm, mean (SD)	15 (9)	-2 (3)
Blood pressure pre-session in mmHg, mean (SD)	126/75 (11/9)	126/74 (12/8)
Blood pressure post-session in mmHg, mean (SD)	128/76 (12/9)	123/73 (12/8)
Standard care physiotherapy received, median [IQR]	2220 [1545 – 2782]	2122 [1540 – 2692]

Comparing the frequencies of intervention sessions in which THR was achieved between patients with and without SAE, no difference between groups was detected (SAE: median 88.9% [IQR 27 to 100] vs. No SAE: median 70% [IQR 27 to 100]).

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Table 3: Baseline characteristics

Baseline characteristics of participants stratified by trial intervention group.*

	Aerobic fitness training group N = 105	Relaxation group N = 95	Study population N = 200
Age in years, mean (SD)	69 (12)	70 (11)	69 (12)
Female sex, n (%)	45 (43)	36 (38)	81 (41)
NIHSS score, median [IQR]‡	9 [5 – 12]	7 [5 – 11]	8 [5 – 12]
Left hemisphere stroke, n (%)	40 (38)	48 (50)	88 (44)
Anterior circulation stroke, n (%)	84 (80)	72 (76)	156 (78)
Ischaemic stroke, n (%)	91 (87)	90 (95)	181 (91)
Treatment with alteplase, n (%)§	34 (37)	27 (30)	61 (34)
Ischemic stroke aetiology§			
LAA, n (%)§	17 (19)	19 (21)	36 (20)
Cardioembolism, n (%)§	18 (20)	18 (20)	36 (20)
Small vessel occlusion, n (%)§	16 (18)	15 (17)	31 (17)
Other aetiology, n (%)§	3 (3)	4 (4)	7 (4)
Undetermined aetiology, n (%)§	34 (37)	28 (31)	62 (34)
Competing etiologies, n (%)§	3 (3)	6 (7)	9 (5)
Pre-existing comorbidities			
Atrial fibrillation, n (%)	23 (22)	23 (24)	46 (23)
Diabetes mellitus, n (%)	32 (31)	31 (33)	63 (32)
Arterial hypertension, n (%)	86 (82)	80 (84)	166 (83)
Hypercholesterolemia, n (%)	43 (41)	37 (39)	80 (40)
History of cerebrovascular event, n(%) [a]	27 (26)	27 (28)	54 (27)
History of cardiovascular disease, n (%) [b]	13 (12)	21 (22)	34 (17)
No. of comorbidities, n (%) [c]			
0	3 (3)	5 (5)	8 (4)
1 – 3	68 (65)	58 (61)	126 (63)
> 3	34 (32)	32 (34)	66 (33)
Smoking‖	18 (17)	11 (12)	29 (15)
Clinical parameters			
Heart rate in beats per minute, mean (SD)	77 (12)	76 (14)	76 (13)
Systolic blood pressure in mmHg, mean (SD)	127 (18)	131 (18)	129 (18)
Diastolic blood pressure in mmHg, mean (SD)	73 (13)	77 (13)	75 (13)
Body mass index in kg/m^2, mean (SD)	26 (4)	26 (4)	26 (4)
Concomitant medication			
Antiplatelets, n (%)	48 (46)	54 (57)	102 (51)
Oral anticoagulation, n (%)	57 (54)	44 (46)	101 (51)
Beta blocker, n (%)	49 (47)	47 (50)	96 (48)
Statins, n (%)	79 (75)	80 (84)	159 (80)

* Patients in the aerobic training group received physical fitness training plus standard care. Patients in the relaxation group received relaxation sessions plus standard care.

† No data available in four patients, because patients were excluded as screening failures prior to intervention start.

‡ Scores on the National Institutes of Health Stroke Scale (NIHSS) range from 0 to 42, with higher scores indicating greater stroke severity. Assessed on day 3-5 after stroke. The NIHSS score of one patient was missing due to missing charts from the acute hospital.

§ Reported proportions of patients treated with alteplase and proportions of stroke aetiology refer only to ischemic stroke patients.

‖ History of smoking was not available in 25 patients.

[a] Cerebrovascular event comprises any occurrence of either stroke or transient ischemic attack

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[b] Cardiovascular disease comprises any occurrence of either coronary artery disease, myocardial infarction or periphery artery disease

[c] Number of comorbidities reflects the load of comorbidities and comprises the number of all documented pre-existing conditions as atrial fibrillation, diabetes mellitus, arterial hypertension, coronary artery disease, transient ischemic attack, stroke, coagulation disorder, periphery artery disease, myocardial infarction, migraine, thyroid disease, tumor, sleep apnoe, amyotrophic lateral sclerosis, multiple sclerosis, morbus Parkinson, hypercholesterolemia, or any other disease.

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Table 4: Characteristics of patients with SAE

Treatment arm	Patient	Type of first SAE	Type of second SAE	Type of third SAE	Time from stroke to intervention start in days	Atrial fibrillation	Diabetes mellitus	Arterial hypertension	History of cerebrovascular	History of cardiovascular	Hypercholesterolemi
Relaxation	1	Cerebrovascular event	Readmission to hospital		10			X		X	
	2	Cerebrovascular event			14			X		X	X
	3	Cerebrovascular event			22			X			
	4	Cerebrovascular event			24		X	X	X		X
	5	Readmission to hospital	Readmission to hospital		NA		X				
	6	Readmission to hospital			40			X			
	7	Readmission to hospital			43	X					
	8	Readmission to hospital			40	X			X		X
	9	Readmission to hospital	Readmission to hospital	Death	21			X	X	X	
	10	Readmission to hospital			9			X	X		X
	11	Death			8						
	12	Death			28		X	X		X	
	13	Death			13		X	X			X
Training	14	Cerebrovascular event	Readmission to hospital	Cerebrovascular event	26			X	X		
	15	Cerebrovascular event	Readmission to hospital		17	X	X	X		X	X
	16	Cerebrovascular event			19		X	X			X
	17	Cerebrovascular event			39	X	X	X			
	18	Cerebrovascular event			53			X		X	
	19	Cerebrovascular event			15	X	X	X	X		X
	20	Cerebrovascular event			37		X	X			
	21	Cerebrovascular event	Readmission to hospital		8			X	X		X
	22	Cerebrovascular event			9			X			X
	23	Readmission to hospital	Readmission to hospital		34	X	X	X	X		
	24	Readmission to hospital	Readmission to hospital		17	X		X	X		
	25	Readmission to hospital			52		X	X		X	
	26	Readmission to hospital			41			X			
	27	Readmission to hospital			47			X			X
	28	Readmission to hospital			39	X		X			
	29	Readmission to hospital			47		X	X	X	X	
	30	Readmission to hospital			28	X					
	31	Readmission to hospital	Readmission to hospital		17	X	X	X			
	32	Readmission to hospital			39	X	X	X	X		X
	33	Readmission to hospital			NA		X	X			
	34	Readmission to hospital			12		X	X			X
	35	Readmission to hospital			14			X			
	36	Readmission to hospital			8						
	37	Readmission to hospital	Cerebrovascular event		11		X	X	X		X
	38	Readmission to hospital			16			X			
	39	Death			24			X		X	X

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Table 5: Departments of acute house readmissions, and respective diagnoses (absolute frequencies)

Hospital ward	Aerobic fitness training		Relaxation	
	No. of participants experienced event	Total number of events	No. of participants experienced event	Total number of events
Cardiology	**7**	**7**	**2**	**2**
Mitral regurgitation		1		0
No new diagnosis		1 *		0
Tachy-Brady Arrhythmia		1		0
Implantation of cardiac pacemaker		1		1
New diagnosis of atrial fibrillation		2 (1*)		0
Cardiac decompensation with pleural effusions		1		0
Ventricular tachycardia		0		1
Neurology	**5**	**5**	**0**	**0**
Traumatic subdural haematoma		1		0
Extra-intracranial bypass		1		0
Confirmation of tandem stenosis of the A. basilaris		1		0
New stroke (incidental finding)		1		0
Giant-cell arteritis		1		0
Gastroenterology	**3**	**3**	**0**	**0**
Urinary tract infection with Hyponatremia		1		0
Refusal to ingest food		1		0
Ischaemic colitis		1		0
Orthopaedics / Traumatology	**2**	**2**	**2**	**2**
Femoral neck fracture		1		2 *
Metatarsal fracture		1		0
Pneumology	**1**	**1**	**1**	**1**
Respiratory insufficiency		1		0
Pneumonia		0		1
Nephrology	**1**	**1**	**2**	**2**
Kidney failure		1		2
Endocrinology	**1**	**1**	**0**	**0**
Suspected insulinoma		1		0
Other	**1**	**1**	**0**	**0**
Deterioration of general condition		1		0
Tumor center	**0**	**0**	**1**	**1**
Bladder carcinoma		0		1
Psychiatry	**0**	**0**	**1**	**1**
Suicidal tendency		0		1
Surgery	**1**	**1**	**0**	**0**
Wound erysipelas		1		0

* SAE prior to intervention

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Table 6: Baseline comparison of patients with SAE compared to patients without SAE between both intervention groups.

Baseline characteristics	Training		Relaxation		IRR (95% CI) for interaction of treatment group and characteristic ‡
	No SAE	SAE	No SAE	SAE	
Socio-demographics	N = 79	N = 26	N = 82	N = 13	
Age in years, mean (SD)	68 (13)	72 (9)	70 (11)	72 (9)	1.00 (0.94 to 1.05)
Female sex, no. (%)	31 (39)	14 (54)	29 (35)	7 (54)	0.58 (0.16 to 1.92)
Initial stroke					
Time since stroke to intervention start in days, median [IQR] #	31 [17 to 37]	24 [15 to 39]	28 [18 to 42]	22 [12 to 31]	1.02 (0.98 to 1.08)
Ischaemic stroke, no. (%)	66 (84)	25 (96)	77 (94)	13 (100)	-
Left hemisphere stroke, no. (%)	34 (43)	6 (23)	45 (55)	3 (23)	2.35 (0.62 to 10.15)
Anterior circulation, no. (%)	20 (25)	5 (19)	18 (22)	4 (31)	0.34 (0.09 to 1.31)
NIHSS, mean (SD) §	9.3 (4.7)	8.4 (5.4)	8.3 (4.4)	8.5 (4.7)	0.98 (0.86 to 1.13)
Preexisting comorbidities					
Atrial fibrillation, no (%)	14 (18)	9 (35)	21 (26)	2 (15)	4.18 (0.94 to 29.82)
Diabetes mellitus, no (%)	19 (24)	13 (50)	27 (33)	4 (31)	2.67 (0.79 to 10.05)
Arterial hypertension, no (%)	62 (79)	24 (92)	71 (87)	9 (69)	7.93 (1.47 to 61.42)
History of cerebrovascular diseases, no (%) [a]	19 (24)	8 (31)	23 (28)	4 (31)	1.21 (0.36 to 4.30)
History of cardiovascular disease, no (%) [b]	8 (10)	5 (19)	17 (21)	4 (31)	0.63 (0.16 to 2.35)
No. of comorbidities, no. (%) [c]					
0	3 (4)	0 (0)	4 (5)	1 (8)	[d]
1 – 3	55 (70)	13 (50)	49 (60)	9 (69)	0.49 (0.14 to 1.62)
> 3	21 (27)	13 (50)	29 (35)	3 (23)	2.29 (0.68 to 8.62)
History of smoking (%)		13 (17)	5 (20)	10 (12)	1 (8)	2.52 (0.39 to 49.79)

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Clinical parameters					
Heart rate in beats per minute, mean (SD)	78 (12)	73 (13)	77 (14)	73 (13)	0.97 (0.92 to 1.02)
Systolic blood pressure in mmHg, mean (SD)	126.1 (17.5)	128.3 (18.2)	130.8 (18.9)	133.5 (15.6)	1.01 (0.98 to 1.04)
Diastolic blood pressure in mmHg, mean (SD)	73.6 (13.6)	71.9 (9.0)	77.0 (13.3)	75.2 (14.5)	1.02 (0.97 to 1.07)
Body mass index in kg/m^2, mean (SD)	25.8 (4.3)	26.4 (4.3)	26.0 (4.2)	25.6 (4.2)	1.01 (0.89 to 1.15)
Pharmaceutical agents, no (%)					
Antiplatelets	36 (47)	12 (50)	46 (59)	8 (73)	0.87 (0.22 to 3.18)
Anticoagulation	37 (49)	20 (83)	39 (50)	5 (46)	3.12 (0.72 to 15.16)
Statins	55 (72)	20 (83)	67 (86)	9 (82)	4.57 (0.91 to 23.30)
β-blocker	30 (40)	16 (67)	39 (50)	6 (55)	1.75 (0.43 to 6.89)
Blood draw					
Full blood count					
Hemoglobin, in mmol/l, median [IQR][1]	8.60 [7.80 to 9.22]	8.15 [7.60 to 8.70]	8.30 [7.70 to 9.20]	8.10 [6.90 to 9.10]	1.23 (0.72 to 2.10)
Hematocrit, in %, median [IQR][1]	40.50 [37.80 to 43.32]	39.00 [36.42 to 41.95]	39.70 [36.60 to 42.90]	36.60 [32.90 to 41.30]	1.04 (0.92 to 1.19)
Erythrocytes, in Tpt/l, median [IQR][1]	4.63 [4.23 to 4.89]	4.42 [4.02 to 4.78]	4.53 [4.14 to 4.90]	4.40 [4.10 to 4.87]	1.15 (0.42 to 3.15)
Leukocytes, in Gpt/l, median [IQR][1]	7.00 [5.88 to 8.22]	7.45 [5.85 to 8.75]	7.20 [6.30 to 8.70]	7.00 [6.00 to 9.00]	0.88 (0.65 to 1.20)
Thrombocytes, in Gpt/l, median [IQR][1]	280 [244 to 334]	253 [192 to 278]	275 [208 to 337]	260 [236 to 308]	1.00 (0.99 to 1.00)
Metabolic parameters					
Glucose, in mmol/l, median [IQR][2]	6.05 [5.38 to 6.96]	6.88 [5.99 to 8.99]	6.05 [5.38 to 7.49]	7.22 [5.66 to 8.83]	1.05 (0.84 to 1.36)
Insulin, in µU/ml, median [IQR][1]	9.95 [7.05 to 14.43]	12.15 [7.67 to 17.60]	9.10 [7.20 to 14.50]	9.00 [5.80 to 12.90]	1.03 (0.98 to 1.11)
HbA1c, in %, median [IQR][1]	5.75 [5.40 to 6.32]	5.95 [5.53 to 6.70]	5.80 [5.50 to 6.50]	5.80 [5.30 to 6.40]	1.95 (0.94 to 4.56)
High density lipoprotein, in mmol/l, median [IQR][3]	1.20 [0.96 to 1.53]	1.29 [1.17 to 1.47]	1.17 [0.96 to 1.43]	1.12 [1.07 to 1.43]	0.32 (0.06 to 1.71)
Low density lipoprotein, in mmol/l, median [IQR][3]	2.30 [1.70 to 3.00]	1.95 [1.70 to 2.63]	2.10 [1.50 to 2.50]	1.90 [1.60 to 2.20]	0.68 (0.37 to 1.29)
Triglycerides, in mmol/l, median [IQR][3]	1.31 [1.05 to 1.68]	1.14 [1.00 to 1.72]	1.30 [1.07 to 1.68]	1.28 [0.80 to 1.72]	1.01 (0.31 to 3.49)

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Lipoprotein a, in mg/dl, median [IQR][4]	24.40 [9.77 to 66.12]	43.65 [12.55 to 78.65]	22.80 [9.45 to 58.70]	23.00 [5.25 to 59.80]	1.00 (0.99 to 1.01)
Kidney					
Creatinine, in µmol/l, median [IQR][3]	80.00 [68.00 to 89.00]	84.00 [70.50 to 100.00]	79.00 [68.00 to 92.00]	81.00 [69.00 to 96.00]	0.99 (0.99 to 1.00)
eGFR, in ml/min, median [IQR][3]	71.00 [58.00 to 94.00]	62.50 [53.50 to 72.25]	69.00 [55.00 to 94.00]	75.00 [52.00 to 82.00]	1.00 (0.98 to 1.03)
Inflammation					
Fibrinogen, in g/l, median [IQR][5]	3.94 [3.10 to 5.01]	4.32 [3.33 to 4.99]	3.87 [3.38 to 4.73]	3.67 [3.07 to 4.06]	1.80 (0.94 to 3.76)
hs-CRP, in mg/l, median [IQR][6]	5.21 [1.66 to 12.90]	5.58 [1.52 to 16.85]	6.26 [2.04 to 13.22]	4.82 [0.98 to 8.38]	1.06 (1.00 to 1.16)
TNF-alpha, in pg/ml, median [IQR][7]	7.70 [6.30 to 10.15]	9.05 [7.65 to 10.50]	9.00 [7.00 to 10.95]	8.45 [6.68 to 11.20]	1.01 (0.88 to 1.17)
IL-6, in pg/ml, median [IQR][8]	3.75 [2.82 to 5.78]	6.05 [3.40 to 8.67]	4.40 [3.00 to 7.35]	4.60 [2.67 to 5.72]	1.04 (0.96 to 1.22)
Hormones					
Cortisol, in nmol/l, median [IQR][3]	163 [130 to 195]	175 [148 to 233]	177 [142 to 208]	154 [134 to 184]	1.01 (1.00 to 1.02)
TSH, in µlU/ml, median [IQR][9]	1.44 [0.86 to 2.10]	2.10 [0.85 to 3.47]	1.38 [0.88 to 2.07]	1.79 [1.23 to 2.36]	0.86 (0.65 to 1.26)

Patients in the training group received physical fitness training plus standard care. Patients in the relaxation group received relaxation sessions plus standard care. eGFR denotes estimated Glomerular Filtration Rate, hs-CRP denotes high-sensitivity C-reactive protein, TNF-alpha denotes Tumor Necrosis Factor alpha, IL-6 denotes Interleukin 6 and TSH denotes Thyroid-stimulating hormone. Incidence Rate Ratios are calculated from unadjusted Poisson regression models with Treatment, patient characteristics and an interaction term of treatment arm with respective patient characteristic.

‡ IRR indicating differential Incidence of SAE in treatment groups by characteristic.

Data are missing for four patients due to SAE prior to first day of intervention.

§ Data are missing for one patient in the relaxation group due to missing charts from the acute hospital.

| History of smoking was not available in 25 patients.

[a] Cerebrovascular event comprises any occurrence of either stroke or transient ischemic attack.

[b] Cardiovascular event comprises any occurrence of either coronary artery disease, myocardial infarction or periphery artery disease.

[c] Number of comorbidities reflects the load of comorbidities and comprises the number of all documented pre-existing conditions as atrial fibrillation, diabetes mellitus, arterial hypertension, coronary artery disease, transient ischemic attack, stroke, coagulation disorder, periphery artery disease, myocardial infarction, migraine, thyroid disease, tumor, sleep apnoe, amyotrophic lateral sclerosis, multiple sclerosis, morbus Parkinson, hypercholesterolemia, or any other disease.

[d] Unable to compute IRR due to low number of cases.

[1] Data are missing for three patients in the training group and for one in the relaxation group.

[2] Data are missing for five patients in the training group and for one in the relaxation group.

[3] Data are missing for two patients in the training group and for one in the relaxation group.

[4] Data are missing for seven patients in the training group and for eight in the relaxation group.

[5] Data are missing for five patients in the training group and for two in the relaxation group.

[6] Data are missing for six patients in the training group and for two in the relaxation group.

[7] Data are missing for four patients in the training group and for three in the relaxation group.

[8] Data are missing for 19 patients in the training group and for 15 in the relaxation group.

[9] Data are missing for five patients in the training group and for four in the relaxation group.

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Exploratory secondary endpoints

Mean hospitalization time was 67 (SD 31) days in both groups and the amount of applied physiotherapy (median 2220 [1545 – 2782] min *vs.* and 2122 [1540 – 2692] min, respectively) was similar in both groups. Hospitalization times at the rehabilitation clinic of both treatment groups showed differences between patients with SAE and patients without SAE (training: 6, 95% CI -10 to 23 days *vs.* relaxation: 22, 95% CI -11 to 56 days). Relative to being released home, patients with an SAE during hospital stay were less likely to be discharged to a nursing home as patients without SAE (OR 0.55, 95% CI 0.07 to 4.50) and more likely to be discharged to another rehabilitation clinic (OR 1.75, 95% CI 0.19 to 15.77). Exploratory analysis revealed that SAE occurrence was not associated with the primary efficacy endpoint maximal walking speed three months after stroke (0.0 m/s 95% CI -0.2 to 0.2) or six months after stroke (0.0 m/s, 95% CI -0.2 to 0.2). In contrast, occurrence of SAE was associated with a lower Barthel-Index (-11 points, 95% CI -19 to -3) at three months, but this association was weaker at six months after stroke (-5 points, 95% CI -12 to 1).

Exploratory Risk factor analyses

The final adjusted model analyzing the association of DM with SAE included treatment arm, DM, β-blocker medication, and the interaction between treatment arm and DM diagnosis (IRR for interaction 7.10, 95% CI 1.56 to 51.24). Post-hoc estimated marginal means of interaction terms equally demonstrated a higher IRR in patients with DM (6.96, 95% CI 1.60 to 30.35) compared to patients without DM (0.98, 95% CI 0.45 to 2.16) when comparing training and control groups. When diabetes mellitus was substituted by HbA1c in the model, a higher risk of SAE was similarly observed in the training group (IRR for interaction: 3.52, 95% CI 1.41 to 9.29).

With regard to AF, the final model analyzing the association with SAE occurrence included treatment, AF, β-blocker medication, and the interaction between treatment arm and AF (IRR for interaction 4.37, 95% CI 0.94 to 31.81). Observed associations remained stable after controlling for age, sex, and NIHSS. Post-hoc calculation of estimated marginal means showed a higher risk in patients with AF (IRR 5.50, 95% CI 1.22 to 24.80) compared to patients without AF (IRR 1.26, 95% CI 0.60 to 2.66) when comparing training and control group.

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Table 7: Model comparison for association of arterial hypertension, diabetes mellitus, atrial fibrillation and HbA1c with SAE.

	Model 1 (model with treatment, hypertension and interaction of treatment and hypertension), Incidence-Rate-Ratio (95%CI)	Model 2 (model with treatment, diabetes and interaction of treatment and diabetes), Incidence-Rate-Ratio (95%CI)	Model 3 (model with treatment, AF and interaction of treatment and AF), Incidence-Rate-Ratio (95%CI)	Model 2.1 (model with treatment, HbA1c and interaction of treatment and HbA1c), Incidence-Rate-Ratio (95%CI)
Intercept	0.03 (0.01 to 0.07) ***	0.02 (0.01 to 0.04) ***	0.02 (0.01 to 0.04) ***	0.01 (0.00 to 0.02) ***
Treatment arm training group	0.44 (0.06 to 2.68)	0.98 (0.44 to 2.19)	1.26 (0.60 to 2.72)	3.02 (1.38 to 7.75) *
Arterial hypertension	0.51 (0.16 to 2.46)			
Diabetes mellitus		0.32 (0.05 to 1.18)		
Atrial fibrillation			0.38 (0.06 to 1.42)	
HbA1c in %				0.27 (0.11 to 0.62) **
Age in years	1.01 (0.97 to 1.04)	1.01 (0.98 to 1.05)	1.01 (0.98 to 1.04)	1.01 (0.98 to 1.04)
Female sex	1.99 (1.06 to 3.69) *	1.90 (1.04 to 3.50) *	1.74 (0.94 to 3.24)	1.73 (0.92 to 3.28)
Stroke severity (NIHSS)	0.98 (0.91 to 1.06)	0.99 (0.92 to 1.07)	0.99 (0.92 to 1.07)	1.00 (0.92 to 1.08)
Beta blocker medication	2.45 (1.26 to 5.07) *	2.27 (1.17 to 4.70) *	2.47 (1.26 to 5.12) *	3.07 (1.52 to 6.62) **
History of smoking				
Waist-to-hip ratio				
Interaction arterial hypertension with treatment group	5.30 (0.76 to 45.55)			
Interaction diabetes with training group		7.10 (1.56 to 51.24) *		
Interaction atrial fibrillation with training group			4.37 (0.94 to 31.81)	
Interaction HbA1c with training group				3.52 (1.41 to 9.29) **
N	188	188	188	184
Pseudo R² (Fixed effects)	24.1%	25.8%	20.9%	49.6%
Pseudo R² (total)	§	§	§	53.1%
AIC	244.8	240.6	244.5	233.7
BIC	273.9	269.7	273.7	262.6

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

§ Computation of random effect variances not possible because some variance equal to zero.

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Table 7 continued (adjustment of Model 2 and 3 for patient-related lifestyle risk factors).

	Model 2.2 (model with treatment, diabetes and interaction of treatment and diabetes adjusted for lifestyle risk factors), Incidence-Rate-Ratio (95%CI)	Model 3.1 (model with treatment, AF and interaction of treatment and AF adjusted for lifestyle risk factors), Incidence-Rate-Ratio (95%CI)
Intercept	0.02 (0.01 to 0.04)*	0.02 *** (0.01 to 0.04)
Treatment arm training group	1.04 (0.46 to 2.32)	1.26 (0.59 to 2.75)
Arterial hypertension		
Diabetes mellitus	0.31 (0.05 to 1.16)	
Atrial fibrillation		0.41 (0.06 to 1.55)
HbA1c in %		
Age in years	1.01 (0.98 to 1.05)	1.01 (0.98 to 1.04)
Female sex	2.03 (1.00 to 4.02) *	1.94 (0.95 to 3.90)
Stroke severity (NIHSS)	0.98 (0.91 to 1.06)	0.99 (0.91 to 1.06)
Beta blocker medication	2.23 (1.13 to 4.65)*	2.29 * (1.15 to 4.80)
History of smoking	0.87 (0.29 to 2.12)	0.87 (0.29 to 2.10)
Waist-to-hip ratio	4.99 (0.06 to 354.23)	8.45 (0.10 to 589.46)
Interaction arterial hypertension with treatment group		
Interaction diabetes with training group	6.18 (1.33 to 45.20) *	
Interaction atrial fibrillation with training group		4.18 (0.88 to 29.22)
N	187	187
Pseudo R² (Fixed effects)	24.3%	20.3%
Pseudo R² (total)	§	§
AIC	237.9	240.3
BIC	273.5	275.8

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12.   Lebenslauf

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

13. Publikationsliste

(Stand November 2021)

Artikel in Fachzeitschriften
Nave AH, **Rackoll T**, Grittner U, Bläsing H, Gorsler A, Nabavi DG, Audebert HJ, Klostermann F, Müller-Werdan U, Steinhagen-Thiessen E, Meisel A, Endres M, Hesse S, Ebinger M, Flöel A. *Physical Fitness Training in Patients with Subacute Stroke (PHYS-STROKE): multicentre, randomised controlled, endpoint blinded trial.* BMJ. 2019 Sep 18;366:l5101.
doi: 10.1136/bmj.l5101.
(Geteilte Erstautorenschaft)

Rackoll T, Neumann K, Passmann S, Grittner U, Külzow N, Ladenbauer J, Flöel A. *Applying time series analyses on continuous accelerometry data-A clinical example in older adults with and without cognitive impairment.* PLoS One. 2021 May 13;16(5):e0251544.
doi: 10.1371/journal.pone.0251544.
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Gorsler A, Grittner U, Külzow N, **Rackoll T**. *Blinding in electric current stimulation in subacute neglect patients with current densities of 0.8 A/m²: a cross-over pilot study.* BMC Res Notes. 2021 Jan 25;14(1):35. doi: 10.1186/s13104-020-05421-7.

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doi: 10.1177/17474930211006286.
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Kufner A, Khalil AA, Galinovic I, Kellner E, Mekle R, **Rackoll T**, Boehm-Sturm P, Fiebach JB, Flöel A, Ebinger M, Endres M, Nave AH. *Magnetic resonance imaging-based changes in vascular morphology and cerebral perfusion in subacute ischemic stroke.* J Cereb Blood Flow Metab. 2021 Oct;41(10):2617-2627. doi: 10.1177/0271678X211010071.

Kirzinger B, Stroux A, **Rackoll T**, Endres M, Flöel A, Ebinger M, Nave AH. *Elevated Serum Inflammatory Markers in Subacute Stroke Are Associated With Clinical Outcome but Not Modified by Aerobic Fitness Training: Results of the Randomized Controlled PHYS-STROKE Trial.* Front Neurol. 2021 Aug 26;12:713018. doi: 10.3389/fneur.2021.713018.

Müller S, Kufner A, Dell'Orco A, **Rackoll T**, Mekle R, Piper SK, Fiebach JB, Villringer K, Flöel A, Endres M, Ebinger M, Nave AH. _Evolution of Blood-Brain Barrier Permeability in Subacute Ischemic Stroke and Associations With Serum Biomarkers and Functional Outcome._ Front Neurol. 2021 Oct 20;12:730923. doi: 10.3389/fneur.2021.730923.

Hair K, Sena E, Wilson E, Currie G, Macleod M, Bahor Z, Sena C, Ayder C, Liao J, Tanriver Ayder E, Ghanawi J, Tsang A, Collins A, Carstairs A, Antar S, Drax K, Neves K, Ottavi T, Chow YY, Henry D, Selli C, Fofana M, Rudnicki M, Gabriel B, Pearl E, Kapoor S, Baginskaite J, Shevade S, Chung A, Przybylska M, Henshall D, Hajdu K, McCann S, Sutherland C, Lubiana Alves T, Blacow R, Hood R, Soliman N, Harris A, Swift S, **Rackoll T**, Percie du Sert N, Waldron F, Macleod M, Moulson R, Low J, Rannikmae K, Miller K, Bannach-Brown A, Kerr F, Hébert H, Gregory S, Shaw I, Christides A, Alawady M, Hillary R, Clark A, Jayasuriya N, Sives S, Nazzal A, Jayasuriya N, Sewell M, Bertani R, Fielding H, Drury B., _Building a Systematic Online Living Evidence Summary of COVID-19 Research._ JEAHIL. 24Jun.2021;17(2):21-6. doi.org/10.32384/jeahil17465

Online Publikationen

Rackoll T, Grittner U, Nave A, Flöel A, Ebinger M. _Physical Fitness in Subacute Stroke (PHYS-Stroke) - Statistical Analysis Plan._ Protocol available at https://doi.org/10.6084/M9.FIGSHARE.5375026.V1

Rackoll T, McCann S, Bannach-Brown A, Sena E, Cruz F, Dirnagl U, Lawrence C . _Systematic review and meta-analysis of the effects of diabetes mellitus, obesity and metabolic syndrome on stroke outcome and treatment efficacy in animal models of ischaemic stroke._ Protocol available at https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=191339

Rackoll T, Bannach-Brown A, Lawrence C, Vojvodic S, Iqbal S, Hobby D, Sena E, Dirnagl U, McCann S. _Systematic review and meta-analysis of the effects of arterial hypertension on stroke outcome and treatment efficacy in animal models of ischaemic stroke._ Protocol available at https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=283853

Kongressbeiträge

2019	_tDCS in der Praxis. Erfahrungen bei Neglect Patienten_ **(Vortrag, DGNKN Berlin)**
2017	_Aerobes Laufbandtraining bei subakuten Schlaganfallpatienten – ist es durchführbar und sicher?_ **(Vortrag, DGNR in Berlin)**

14.   Danksagung

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