Introduction
Type 2 diabetes mellitus (T2DM) is associated with an increased risk of developing neurodegenerative diseases [1]. Inflammatory, oxidative and metabolic changes in T2DM patients can promote brain insulin resistance, mitochondrial dysfunction or accumulation of neurotoxic beta-amyloid oligomers in the diabetic brain, leading to neuronal cell death and cognitive decline [2].

Studies provide evidence that physical activity can positively influence brain health and cognitive function [2, 3]. In this context, the brain-derived neurotrophic factor (BDNF) is the most prominent neurotrophin and has been shown to be a key molecule for increased neuronal plasticity and exercise-induced neurogenesis [4]. Thus, experiments with rats have shown that blocking BDNF inhibits the beneficial effects of exercise training on cognitive function [5]. An extensive meta-analysis involving original studies with healthy humans concludes that acute high intensity exercise as well as moderate intensity exercise can increase BDNF levels in blood, although the values of circulating BDNF do not remain elevated and decrease relatively quickly during recovery [6]. To date, nothing is known about the effects of acute exercise on BDNF levels in T2DM patients. The molecule is released from the brain, but also expressed in other tissues, e.g., in skeletal muscle, retina, kidney or prostate [7]. Rasmussen et al. [8] showed that the brain can contribute about 70–80% of circulating BDNF in healthy humans following acute exercise. Increases in BDNF levels, which are repeatedly generated by acute exercise during a training intervention, are likely to help improve brain plasticity considering that daily intravenous BDNF bolus injections have been shown to induce neurogenesis in rat hippocampus [9] and that increases in BDNF activate appropriate signaling pathways in cultured rat hippocampal neurons [10]. It is also interesting that cognitive stimulation alone may increase neuronal plasticity via BDNF, because it has been...
demonstrated that BDNF expression is rapidly induced in rat hippocampus during contextual learning [11].

Not only BDNF, but other factors may also play an important role in increasing neurogenesis and neuroplasticity: the vascular endothelial growth factor (VEGF) (expressed in several cells and tissues, e.g., in skeletal muscle, endothelial cells, macrophages and glial cells) [12] and the insulin-like growth factor (IGF)-1 (also expressed in several cells and tissues, e.g., in skeletal muscle, the liver, neurons and glial cells) [13]. Some human studies indicate that acute exercise can increase VEGF and IGF-1 in the periphery [14, 15]. Both molecules can pass the blood-brain-barrier [3]. There is also evidence from animal studies that acute exercise can transiently increase VEGF levels directly in the brain [12]. VEGF and IGF-1 may contribute to the induction of neurogenesis, as it has been demonstrated that blocking VEGF and IGF-1 entry to the brain prevents exercise-induced neurogenesis in the hippocampus of rats [16, 17]. Furthermore, VEGF and IGF-1 can help modulate vascular remodeling, synaptic plasticity, synaptic density and neurotransmission [18].

"Exergaming" (the combination of exercise and video gaming involving additional cognitive stimulation) has attracted increasing attention among young as well as elderly persons [19].

This study aims to analyze whether acute exercise can increase BDNF, VEGF and IGF-1 levels in elderly T2DM patients (> 65 years) and whether there is a difference between the effects of traditionally recommended submaximal cycling and exergaming at the same rating of perceived exertion on these neurotrophic factors.

Materials and Methods

Patients

All subjects were recruited via a newspaper advertisement. 8 elderly overweight/obese non-insulin-dependent type 2 diabetic men (71 ± 4 years) participated in the study (▶ Table 1). The duration of their diabetic disease had been 6 ± 3 years (self-report). The inclusion criteria required the subjects to be non-smokers and free of diabetic nephropathy, neuropathy, retinopathy and/or any other cardiovascular complications (apart from hypertension in 8 patients)/chronic diseases. All patients visited their diabetologists regularly. All subjects were taking medications during the investigation period (anti-diabetic drugs n = 6, anti-hypertensive/cardio-protective drugs n = 8, others: drugs against hyperlipidemia n = 4, drugs against hypothyroidism n = 2, drugs against benign prostatic enlargement n = 2). It was determined by questionnaire that none of the subjects had regularly exercised (> 1 time per week) in the last year before the commencement of the study.

Study design

The protocol for the research project was approved by a suitably constituted Ethics Committee of the German Sport University before the investigation. It conformed to the provisions of the Declaration of Helsinki. Written informed consent was obtained from all subjects.

The study design is presented in ▶ Fig. 1. Prior to the randomized crossover study, blood was collected from the subjects in the fasting state to determine their glycemic control and blood lipid profile. Furthermore, the subjects participated in an endurance test (WHO cycling step test) to determine whether they could perform moderate to vigorous exercise without any health risks. The test was performed on the upright bike “Ergometrics900” (Ergoline, Bitz, Germany) coupled to an ECG (“Ergoscript EK3012”, Ergoline). Starting at 25 W, intensity gradually increased by 25 W every 2 min. Subjects were tested with the following stopping criteria: muscular exhaustion, angina pectoris, ischemia, paleness, cyanosis, arrhythmia, respiratory insufficiency, hypertension (systolic blood pressure: > 250 mmHg or diastolic blood pressure > 115 mmHg), aberration, dizziness and/or coordination problems. 8 subjects passed the test (maximum workload during the test: 137 ± 36 W) and were enrolled in the study.

The randomized 2 × 2 crossover study (AB, BA: A = cycling, B = exergaming) started 1 week after the endurance tests. Venous blood was collected pre-as well as immediately post-30-min cycling and exergaming to determine circulating BDNF, VEGF and IGF-1.

Randomized crossover study: 30-min cycling and 30-min exergaming

The subjects conducted 30 min of cycle exercise on the upright bike “Ergometrics900” with constant revolutions per minute [rpm = ~65].

The subjects also played interactive video games using the “Wii Fit Plus” system (Nintendo, Kyoto, Japan) for 30 min following a brief theoretical introduction (explanation of the games). Games combining physical activity and complex cognitive stimulation were selected for the study (“Basic Run Plus”: jogging while memorizing details of the landscape (players have to answer questions at the end of the game); “Obstacle Island”: walking (on a balance board) while avoiding collisions with obstacles; “Island Cycling”: virtual cycling (walking on a balance board and using a handheld device to determine the direction) while collecting flags in the landscape). The games changed every 5 min to increase the subjects’ motivation and to ensure a high level of concentration.

The exercise intensity in both experimental runs (cycling, exergaming) was determined using the original Borg Rating of Perceived Exertion (RPE) scale (reaching from 6 to 20). Patients were to exercise at a submaximal intensity of BORG RPE scale 14–15 dur-
ing cycling as well as during exergaming. Exercise intensities were adapted accordingly. Cycling load was increased/decreased or patients were asked to increase/decrease the velocity of walking/jogging during exergaming. Patients wore heart rate monitors ("FT1", Polar, Büttelborn, Germany) to measure their heart rates during the exercise sessions. Furthermore, capillary blood was taken from their earlobes at the end of each exercise session to determine their capillary blood lactate value. Blood lactate was analyzed using the "Biosen S-line Lab + " system (EKF Diagnostics, Cardiff, United Kingdom).

The subjects were tested with an interval of at least 7 days between the measurements. They were always tested at the same time of day. The patients had been instructed to not engage in any physically exhausting activities during the duration of the study and to not eat or consume any drinks containing carbohydrates 2 h before the measurements. The subjects were furthermore asked to maintain their dietary habits and/or medication intake for the duration of the study.

Measurement of serum neurotrophic factor levels

Blood was collected using the Vacutainer blood withdrawal system of Becton Dickinson (Franklin Lakes, New Jersey, USA). After storage at room temperature for ~15 min, the blood samples were centrifuged at 3,000 rpm for 10 min. The serum was stored at ~80°C. The following enzyme-linked immunosorbent assay (ELISA) kits from R+D systems (Minneapolis, Minnesota, USA) were used for the quantification of serum molecules: BDNF (article number: DBD00), VEGF (DVE00), IGF-1 (DG100). All measurements were performed in duplicate and the individual mean values were used for further analyses.

Statistical analyses

Data are presented as mean values ± standard deviations (SD). Statistical analyses were carried out using the "SPSS 19.0" software (SPSS Incorporation, Chicago, Illinois, USA). Non-parametric (rank-based) hypotheses tests were used throughout as normality of continuous data distributions seemed to be questionable (skewness of data, outliers). Pairwise comparisons were analyzed using the Wilcoxon test. Significance was considered at p-value ≤ 0.05.

Results

Heart rate and lactate values

The participants’ heart rates were measured every 5 min during exercise and the mean value was calculated. The mean heart rate was 113 ± 18 beats/min during cycling and 108 ± 18 beats/min during exergaming. The heart rates did not differ significantly between the 2 experimental runs (p > 0.05), while the lactate values between cycling and exergaming differed significantly (cycling: 3.7 ± 1.1 mmol/l, exergaming: 2.5 ± 1.2 mmol/l, p = 0.043).

Neurotrophic factor levels pre- and post-exercise

All BDNF, VEGF and IGF-1 values were detectable with the ELISA kits used. A high inter-individual range in basal BDNF, VEGF and IGF-1 levels was evident in the diabetic subjects (BDNF: 16–65 ng/ml, VEGF: 117–525 pg/ml, IGF-1: 97–190 ng/ml). BDNF levels increased significantly from pre- to post-cycling (+20%, p = 0.017), but did not increase significantly during exergaming. VEGF levels were increased significantly post-cycling (+14%, p = 0.012), but did not increase through exergaming. IGF-1 did not change during cycling or exergaming (▶ Fig. 2).

Discussion

BDNF, VEGF and IGF-1 have been demonstrated to mediate the effects of exercise on neuronal plasticity and to be involved in neurogenesis [4, 20]. They might thus be important molecules for increasing cognitive function and memory. As there is growing experimental evidence that repeated transient increases in these mol-
Exercise is in line with the results of other studies involving healthy individuals. The observed increase in BDNF levels in the T2DM men post-exercise was significant, while exergaming at the same rating of perceived exertion (BORG RPE scale: 14–15) did not.

The present study revealed that submaximal exercise can increase important neurotrophic factors (BDNF, VEGF) in elderly T2DM patients. However, submaximal cycling increased BDNF and VEGF significantly, while exergaming at the same rating of perceived exertion (BORG RPE scale: 14–15) did not.

The observed increase in BDNF levels in the T2DM men post-exercise is in line with the results of other studies involving healthy subjects [6]. Nofuji et al. [21] demonstrated that BDNF was markedly increased in healthy subjects following moderately intense endurance exercise with the same duration.

Lactate has been shown to be an important trigger for BDNF release. Schiffer et al. [22] have demonstrated that lactate infusion at rest increases BDNF blood concentration in healthy humans. In line with this observation, it has been discussed that more intense exercise with a higher lactate production can increase BDNF to a higher extent than exercise with lower intensities [6]. Specifically, Nofuji et al. [21] reported that 30-min cycling at 60% of maximal oxygen consumption increased BDNF in healthy subjects significantly, while cycling at 40% did not. Considering that the attained lactate concentration might be associated with the amount of released BDNF, it is possible that exergaming with the intensity used in the present study is insufficient to increase BDNF levels reliably due to the fact that the measured lactate values were relatively low in the T2DM patients post-exergaming compared to values post-cycling. Higher lactate values during cycling are most likely attributed to a higher local activity of leg muscles.

Although lower lactate levels were achieved in the T2DM men through exergaming, exergaming has been hypothesized to be at least similar or superior to cycling in increasing BDNF levels due to higher cognitive stimulation. In this context, BDNF expression has been found to be rapidly induced in rat hippocampus during contextual learning [11]. However, additional cognitive stimulation obviously did not contribute to increasing the BDNF levels in the T2DM patients during moderate intensity exergaming.

VEGF and IGF-1 are also neurotrophic factors that mediate neurogenesis [16, 17]. VEGF levels were increased significantly in the T2DM patients post-cycling, and they have already been shown to be increased in healthy trained and untrained men following moderate endurance exercise [23]. Local tissue hypoxia as well as mechanical forces on skeletal muscle and vessels can be significant stimuli for the release of VEGF [24]. It can be speculated that VEGF was not increased post-exergaming due to the fact that oxygen delivery was too high during exergaming (no strong increases in lactate levels underline this assumption) and/or that mechanical forces were not as strong as during cycling. IGF-1 increases have been observed in healthy subjects following low and high intensity resistance exercises, but also following endurance exercises [25, 26]. However, study results are inconsistent and some studies exist showing no change in serum/plasma IGF-1 levels in healthy subjects post-exercise [27]. IGF-1 is produced mainly in the liver with the growth hormone (GH) as its principal regulator [28]. As insulin can increase the hepatic GH receptor availability, many patients with T2DM (and hyperinsulinemia) show augmented basal levels of IGF-1 [28]. Consequently, it might be possible that exercise-induced changes in IGF-1 are attenuated in these patients, and it cannot be excluded that basal IGF-1 levels were increased in the patients involved in the present study.

The effects of exercise on the neurotrophic factors that have been observed in this study as well as the findings from animal experiments that have examined their role in neurogenesis and neuroplasticity, imply that regular physical activity can be beneficial for T2DM patients to prevent or fight neurodegenerative diseases. Future studies are necessary to determine the optimal dose-effect...
relationship between exercise duration/intensity and increases in neurotrophic factors in T2DM patients.

One clear limitation of the present pilot study is the low number of participants. However, due to the complexity and novelty of the study and the special characteristics of the subjects (elderly T2DM patients, >65 years), the study results should be of particular interest.

Conclusions

It can be concluded that acute submaximal exercise can increase neurotrophic factors (BDNF, VEGF) in elderly T2DM patients, depending on exercise mode. Whether the exercise-induced up-regulation of neurotrophic factors can indeed increase neurogenesis and neuroplasticity and improve cognitive function in T2DM patients in the long term requires further research.

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Conflict of interest

The authors have no conflict of interest to disclose.

References