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Effects of a 12-week alpine skiing intervention on endothelial progenitor cells, peripheral arterial tone and endothelial biomarkers in the elderly

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ABSTRACT

Objective: Endothelial dysfunction occurs early during atherogenesis and it can be normalized by exercise training. Unfortunately, patients' compliance with exercise prescription remains low, often because the given choices do not appeal to them. In Alpine regions, skiing is a popular mode of exercise, and therefore we set out to assess whether it can induce antiatherogenic effects.

Methods: We randomized 42 subjects into a group of 12 weeks of guided skiing (intervention group, IG, n = 22; 12 males/10 females; age: 66.6 ± 2.1 years) or a control group (CG, n = 20; 10 males/10 females; age: 67.3 ± 4.4 years). Early (CD3−CD34+CD45+) and late endothelial progenitor cells (EPCs; CD45dimCD34+KDR+ peripheral blood mononuclear cells, PBMCs), peripheral arterial tonometry and endothelial biomarkers were assessed at the beginning and end of the study.

Results: In the IG, participants completed 28.5 ± 2.6 skiing days at an average heart rate of 72.7 ± 8.5% of their maximum heart rate. Changes in early (IG: +0.001 ± 0.001% PBMC; CG: −0.001 ± 0.001% PBMC; IG vs. CG: $p < 0.001$) but not late EPCs differed significantly. Changes in peripheral arterial tone differed significantly between IG (Reactive Hyperemia Index: +0.18 ± 0.76) and CG (−0.39 ± 0.85; $p = 0.045$), as did homocysteine (IG: −1.3 ± 1.3 μmol/l; CG: −0.4 ± 1.4 μmol/l; $p = 0.037$) while other endothelial biomarkers remained essentially unchanged.

Conclusions: This study shows that skiing induces several beneficial effects on markers of atherogenesis including EPCs, peripheral arterial tone and homocysteine. Our findings suggest that recreational alpine skiing may serve as a further mode of preventive exercise training, which might result in improved compliance with current recommendations.

1. Introduction

Endothelial dysfunction is an early stage of atherosclerosis, which is induced by several cardiovascular risk factors [1], and is associated with progression of cardiovascular diseases [2] as well as cardiovascular events [3]. Exercise training exerts a panoply of antiatherogenic effects [4] and has been shown to reverse endothelial dysfunction [5,6]. Despite an abundance of scientific research attesting exercise training a leading role in disease prevention, implementation in target groups of sedentary subjects remains difficult often due to a lack of appealing physical activities, which are the prerequisite for improved adherence. Alpine skiing is performed by millions of recreational skiers in mountainous regions and may be an attractive mode of physical activity for many locals and tourists alike [7].

In order to assess the effects of Alpine skiing on endothelial function, we measured flow mediated vasodilation by peripheral arterial tonometry [8]. Also, we measured proatherogenic markers as well as endothelial progenitor cells (EPCs), since they are all linked to the progression of cardiovascular diseases due to their role in plaque formation [9,10]. Although several definitions exist for EPCs with respect to their surface

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markers, a general consensus has been reached for subdividing EPCs in early (CD3 − CD34 + CD45 + peripheral blood mononuclear cells [PBMCs]) and late (CD45dimCD34 + KDR + PBMCs) EPCs [11,12]. Decreased numbers of EPCs were shown to correlate with cardiovascular risk factors, disease progression and prognosis of coronary artery disease [13,14], whereas beneficial changes in the cardiovascular risk profile improved the number and activity of EPCs [15,16].

Early EPCs are adherent mononuclear cells that emerge after 4–7 days of culture of PBMC on fibronectin-coated plates in an endothelial cell medium. They do not differentiate into endothelial cells, nor incorporate in an endothelial network, but they significantly induce endothelial network formation in vitro and vascular repair in vivo by paracrine release of angiogenic cytokines. Late EPCs are derived from adherent PBMC, cultured in an endothelial medium for 6–21 days. Colonies from these cells display a cobblestone morphology and the cells spontaneously form blood vessels in vivo [17].

It was the aim of this study to investigate, whether Alpine skiing may play a potential role in the prevention of disease progression by inducing positive changes in endothelial markers, early and late EPCs and peripheral vascular tone in elderly recreational skiers.

2. Material and methods

2.1. Study design and population

The design of this study has been previously reported elsewhere [18]. Briefly, the study population was randomized into an intervention group (IG, n = 22; age: 66.6 ± 2.1) or a control group (CG, n = 20; age: 67.3 ± 4.4). The IG performed guided skiing for 12 weeks, with an average of 28.5 ± 2.6 days and 3.5 h/day of skiing, while the CG was asked to maintain their sedentary life style, and to refrain from skiing during the course of the study. All parameters were measured PRE and POST intervention. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee (The Ethics Committee of the University of Salzburg) and written informed consent was obtained from each patient. Study registration: https://clinicaltrials.gov/show/NCT01248910.

2.2. Early and late EPC isolation, specific surface labeling, and flow cytometry

Blood samples were collected in EDTA (ethylenediaminetetraacetic acid) tubes. Within 1 h, PBMCs were separated via density gradient centrifugation and an FcR-blocking reagent was added for 10 min of incubation. Thereafter, incubation with the following specific antibodies was performed (10 μl per 10^7 cells): CD 3 (Fluorescin isothiocyanate; BD Pharmingen, New Jersey, USA), CD34 (Phycoerythrin; BD Pharmingen, New Jersey, USA), CD45 (Peridinin chlorophyll protein; BD Pharmingen, New Jersey, USA), and KDR/anti-VEGFR2 (Allophycocyanin, mouse IgG1, R&D Systems, Minneapolis, USA).

The ISHAGE sequential strategy [12,19] was used for flow cytometry (FACSCalibur cytometer; Becton Dickinson, New Jersey, USA). Briefly, late EPCs (CD45dimCD34 + KDR + PBMCs) were measured and were expressed as percentage of total PBMCs. Early EPCs (CD3 − CD34 + CD45 + PBMCs) were measured using a plotting of CD34/CD45 and double positive cells were then plotted against CD3 to exclude T-cells. Each sample was analyzed (using FlowJo X, Tree Star Inc., Oregon, USA) with the same gating matrix to eliminate intra-observer variability and to improve reproducibility. EPCs were available for 10 subjects each in IG and CG.

2.3. Peripheral arterial tone

Peripheral arterial tone (PAT) was evaluated after a reactive hyperemia challenge with a non-invasive disposable probe at the fingertip (Endo PAT 2000, Itamar Medical, Israel). The reactive hyperemia procedure consists of a 3–10 min baseline recording, 5 min of blood flow occlusion in one arm by using an upper arm blood pressure cuff, and 10 min of recording after cuff release. In a healthy endothelium a post occlusion increase of the PAT signal amplitude is expected. The RHI (Reactive Hyperemia Index) is the ratio between the post- to pre-occlusion average signal size, normalized to the signal of the control arm. In endothelial dysfunction, the post-occlusion PAT signal remains essentially unchanged as compared to the pre-occlusion PAT signal, and consequently the RHI is different to one measured in a healthy endothelium [20].

2.4. Endothelial markers

Endothelial markers like homocysteine, E-selectin, endothelin-1, VCAM-1, ICAM-1 and hs-CRP have previously been measured [21] and are now analyzed to assess potential interplay with EPCs and peripheral vascular tone.

2.5. Statistical analysis

Descriptive statistics are presented as means ± standard deviation (SD). Statistical significance was set at $p < 0.05$. Data were tested for normal distribution using the Shapiro–Wilk test. Intra- and intergroup comparisons of normally distributed parameters were made by paired t-test and unpaired t-tests, and of non-normally distributed parameters by Wilcoxon-signed rank test and Mann–Whitney-U test respectively. Statistical calculations were performed with IBM®SPSS® Statistics Version 21 (SPSS Inc., an IBM Company Headquarters, Chicago, USA).

3. Results

3.1. Baseline characteristics and skiing intervention

In total, 47 subjects participated in the study (IG: n = 27; CG: n = 20), and 42 completed the study (IG: n = 22; CG = 20) [18]. Baseline characteristics are presented in Table 1. In the IG, participants completed 28.5 ± 2.6 skiing days (skiing time: 67.6 ± 7.2 min/d; lift and resting time: 117.3 ± 16.5 min/d, break time: 22.6 ± 18.5 min/d) at an average heart rate of 72.7 ± 8.5% of the maximum heart rate and in sum 4885 ± 816 m (downhill) per skiing day.

3.2. EPCs

EPCs were available in 20 patients (10 in IG and 10 in CG). Changes in early EPCs in the IG (+0.001 ± 0.001%) and the CG (−0.001 ± 0.001%) differed significantly (IG vs. CG: $p < 0.001$). Late EPCs did not change in IG (+0.005 ± 0.036%) and CG (−0.022 ± 0.062%; IG vs. CG: p = 0.235), see Fig. 1 and Table 2.

3.3. Peripheral arterial tone

Peripheral arterial tone changes differed significantly between IG (Reactive Hyperemia Index: +0.18 ± 0.76) and CG (−0.39 ± 0.85; p = 0.045), see Fig. 1 and Table 2.

Table 1
Baseline characteristics.

	IG	CG	P
n=	22	20	
m/f	12m/10f	10m/10f	0.768
age [years]	66.6 ± 2.1	67.3 ± 4.4	0.547
weight [kg]	79.4 ± 13.7	73.7 ± 12.6	0.169
height [m]	1.71 ± 0.09	1.70 ± 0.08	0.672