

# Effect of 24 Sessions of High-Intensity Aerobic Interval Training Carried out at Either High or Moderate Frequency, a Randomized Trial

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## Abstract

**Purpose:** The training response of an intensified period of high-intensity exercise is not clear. Therefore, we compared the cardiovascular adaptations of completing 24 high-intensity aerobic interval training sessions carried out for either three or eight weeks, respectively.

**Methods:** Twenty-one healthy subjects ( $23.0 \pm 2.1$  years, 10 females) completed 24 high-intensity training sessions throughout a time-period of either eight weeks (moderate frequency, MF) or three weeks (high frequency, HF) followed by a detraining period of nine weeks without any training. In both groups, maximal oxygen uptake ( $VO_{2max}$ ) was evaluated before training, at the 9<sup>th</sup> and 17<sup>th</sup> session and four days after the final 24<sup>th</sup> training session. In the detraining phase  $VO_{2max}$  was evaluated after 12 days and thereafter every second week for eight weeks. Left ventricular echocardiography, carbon monoxide lung diffusion transfer factor, brachial artery flow mediated dilatation and vastus lateralis citrate maximal synthase activity was tested before and after training.

**Results:** The cardiovascular adaptation after HF training was delayed compared to training with MF. Four days after ending training the HF group showed no improvement ( $+3.0\%$ ,  $p = 0.126$ ), whereas the MF group reached their highest  $VO_{2max}$  with a  $10.7\%$  improvement ( $p < 0.001$ ; group difference  $p = 0.035$ ). The HF group reached their highest  $VO_{2max}$  ( $6.1\%$  increase,  $p = 0.026$ ) twelve days into the detraining period, compared to a concomitant reduction to  $7.9\%$  of  $VO_{2max}$  ( $p < 0.001$ ) above baseline in the MF group (group difference  $p = 0.609$ ).

**Conclusion:** Both HF and MF training of high-intensity aerobic exercise improves  $VO_{2max}$ . The cardiovascular adaptation following a HF programme of high-intensity exercise is however delayed compared to MF training.

**Trial Registration:** ClinicalTrials.gov NCT00733941.

**Citation:** Hatle H, Støbakk PK, Mølmen HE, Brønstad E, Tjønnå AE, et al. (2014) Effect of 24 Sessions of High-Intensity Aerobic Interval Training Carried out at Either High or Moderate Frequency, a Randomized Trial. PLoS ONE 9(2): e88375. doi:10.1371/journal.pone.0088375

**Editor:** Conrad P. Earnest, University of Bath, United Kingdom

**Received:** September 13, 2013; **Accepted:** January 1, 2014; **Published:** February 7, 2014

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**Funding:** The present study was supported by grants from the K.G. Jebsen Foundation, Norwegian Council of Cardiovascular Disease, The Norwegian Research Council, and Funds for Cardiovascular and Medical Research at St Olav's University Hospital, Trondheim. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

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## Introduction

Maximal oxygen uptake ( $VO_{2max}$ ) is considered the best measure of aerobic capacity and an increase in  $VO_{2max}$  is the most common way of demonstrating an effect of aerobic exercise training [1]. The most efficient exercise intensity for improving  $VO_{2max}$  has been found to be close to maximal heart rate ( $HR_{max}$ ) in both healthy subjects [2] as well as in different patient groups [3–5]. Interval training is an effective way of performing high-intensity aerobic exercise, and our research group have previously demonstrated that high-intensity interval exercise at 90–95% of

$HR_{max}$ , performed 2–3 times per week is superior to continuous moderate exercise at 60–70% of  $HR_{max}$  for improving  $VO_{2max}$  when total exercise volume is equalized [6].

The knowledge of how a short and intensified period of high-intensity exercise influences the progression of  $VO_{2max}$  is however less clear. Such block periodization could provide a highly time-efficient method for improving  $VO_{2max}$  within a very short time-period [7]. A recent study of Swiss alpine junior skiers found that 15 high-intensity exercise sessions at 90–95% of  $HR_{max}$  performed over 11 days improved  $VO_{2max}$  by 6% when measured seven days after completing training [8]. However, two earlier studies have

reported a decrease in  $\text{VO}_{2\text{max}}$  and work performance after two weeks of highly intensified training in competitive cyclists [9,10]. This indicates that the assessment of  $\text{VO}_{2\text{max}}$  may be dependent of when the measurements are taking place after completing a highly intensified training period. Also the cardiovascular and pulmonary adaptations have shown to be depressed immediately after intensified exercise [11,12]. Therefore, the aim of the present study was to investigate the progression of aerobic adaptation in young, healthy individuals completing 24 interval training sessions carried out over either 3 weeks or 8 weeks, respectively. A secondary aim was to study the cardiovascular, pulmonary, endothelial and skeletal muscle adaptation induced by the two training programmes.

## Methods

### Participants

Twenty-one students ( $23.0 \pm 2.1$  years, 10 females) were recruited from the Norwegian University of Science and Technology in Trondheim, Norway. Inclusion criteria were; healthy, non-smokers,  $\text{BMI} \leq 27$ , age  $\leq 30$  years, not practising aerobic training more than twice per week and not exceeding a  $\text{VO}_{2\text{max}}$  of 50 and 60  $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for women and men, respectively. The subjects were matched by gender and randomly assigned to a high frequency (HF) ( $n = 11$ ; 6 females), or a moderate frequency (MF) ( $n = 10$ ; 4 females) exercise group (Figure 1). The unit of Applied Clinical Research at the university carried out all randomization procedures to ensure complete blinded randomization. All participants signed written informed consent. The study protocol was approved by the regional committee for medical research ethics (REK midt 4.2008.1755) and registered by ClinicalTrials.gov (Identifier NCT00733941). The protocol for this trial and supporting CONSORT checklist are available as supporting information; see Checklist S1 and Protocol S1. The study was carried out from September 2008 to January 2009.

### Exercise Protocol

The training period consisted of 24 training sessions carried out over a time span of either eight or three weeks for the MF group and the HF group, respectively. A subsequent detraining period consisted of nine weeks without aerobic training, except necessary daily activities. Both groups performed high-intensity aerobic training running uphill on a treadmill, with individually adjusted speed and inclination, supervised by a personal trainer. Each training session consisted of 10 minutes warm-up followed by 4 × 4-minute intervals at 90–95% of  $\text{HR}_{\text{max}}$ , alternating with 3-minute of active recovery at 70% of  $\text{HR}_{\text{max}}$ , ending with a 5-minute cool-down, giving a total exercise time of 40 minutes per session. Heart rate (HR) was monitored by HR recorders (Polar, Finland) to ensure that all training sessions was carried out at definitive intensities.

### Maximal Oxygen Uptake Testing

Maximal oxygen uptake was tested using an individualized graded step incremental test on a treadmill (Woodway, PPS 55). After a 10 minutes warm-up, a facemask was placed on the subjects for metabolic measurements using a Metamax II (Cortex, Leipzig, Germany), where incline was constant at 10% and speed was increased by 1  $\text{km} \cdot \text{h}^{-1}$  every minute. Tests were terminated when candidates reached a  $\text{VO}_2$  plateau that remained stable despite increased work load, i.e.  $\text{VO}_2$  did not increase more than 2  $\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  despite increased work load, and a respiratory exchange ratio (RER)  $> 1.10$ .  $\text{VO}_{2\text{max}}$  was measured

before study started (pre-test) and four days after the 24<sup>th</sup> and final training session (post-test).  $\text{VO}_{2\text{max}}$  was also tested during the training period, i.e. at the 9<sup>th</sup> and 17<sup>th</sup> training session, and after these tests the subjects performed 3 × 4-minute intervals. In the detraining phase the subjects were tested 12 days after the last training session and thereafter every second week until the end of the eight week detraining phase. Thus, a total of eight  $\text{VO}_{2\text{max}}$ -tests were conducted for both groups (Figure 2).

### Echocardiographic Measurements

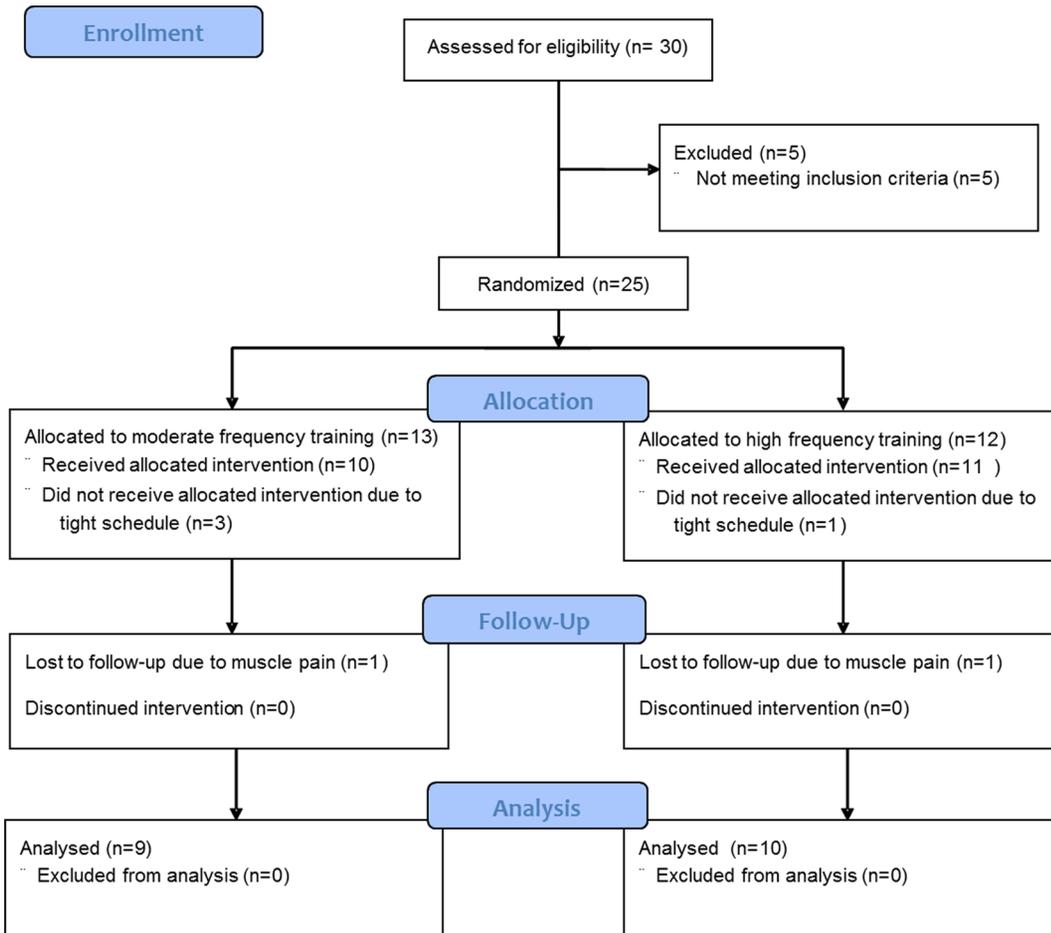
Echocardiography at rest was performed at baseline and repeated six days after the training period ended. Measurements were also repeated after the nine weeks of detraining. Complete echocardiographic examinations were performed using a Vivid 7 scanner (GE Vingmed Ultrasound, Horten, Norway) with a phased-array transducer (3MS probe). Three cine loops from the three standard apical planes (four-chamber, two-chamber and long-axis) as well as short-axis (basal, mid-papillary and apical) were recorded in gray scale harmonic mode and tissue Doppler mode. B-mode recordings were done with an average of 53 frames per second and tissue Doppler with 154 frames per second. Measurements and calculations obtained were in accordance to standard procedures recommended by the American Society of Echocardiography. LV volumes and ejection fraction (EF) were calculated from apical recordings by modified biplane Simpson's method. Inflow velocity of the left ventricle was measured by pulsed Doppler with the sample volume at the tips level of mitral valves. Measurements included early diastolic filling (E) and late diastolic filling (A). Stroke volume (SV) and cardiac output (CO) was calculated by Doppler flow measurement in the left ventricular outflow tract (LVOT). Tissue Doppler recordings of the mitral annulus were obtained from the septal and lateral points in four-chamber and in the anterior and inferior points in two-chamber views. Peak annulus velocity in systole (S'), early diastole (e') and late diastole (A') were measured as average of the four points.

### Lung Function

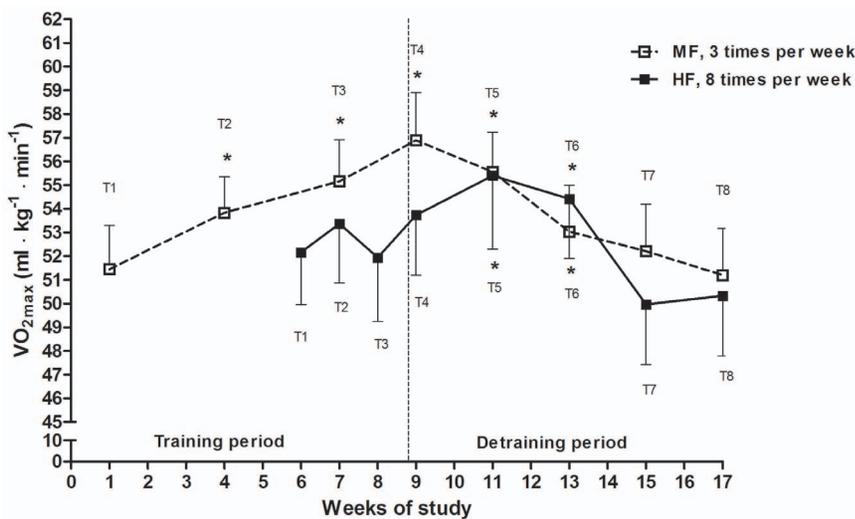
Lung function testing was performed before and four days after the training period. Flow-volume spirometry [13] was performed with the Master Screen Pneumo Spirometer (Jaeger, GmbH & Co, KG). The spirometry was calibrated daily, and the highest of two measurements with  $< 5\%$  variation was recorded [14]. Transfer Factor of the Lung for Carbon Monoxide (TLCO) was measured by the "single breath"-method with gas mixture of 10% helium, 0.3% carbon monoxide, 21% oxygen and 68.7% nitrogen [13] with the Master Screen Diffusion (Jaeger, GmbH & Co, KG). All lung function tests were performed at sea level at room temperature (20–22°C).

### Endothelial Function

Endothelial function was measured before and after the training phase. The measurements were done 1–3 weeks prior to study start and 10 days after the last training session for both groups. Endothelial function was measured as flow-mediated dilatation (FMD) with high-resolution vascular ultrasound using a 14 MHz echo Doppler probe (Vivid 7 System, GE Vingmed Ultrasound, Horten, Norway) according to guidelines [15]. The procedures for measuring FMD and blood pressure were recently published by our group [16]. All ultrasound images were analyzed in random order with EchoPACtm (GE Vingmed Ultrasound AS) by a blinded investigator.



**Figure 1. CONSORT flow diagram of the study design.**  
doi:10.1371/journal.pone.0088375.g001



**Figure 2. Mean values and standard error of the mean of VO<sub>2max</sub> for the two groups during the training and the detraining period are shown.** Vertical dotted line states the last day of training. Confer Table 2 for statistics and exact values at test points (T1–T8). \* Indicates significant differences from baseline within groups.  
doi:10.1371/journal.pone.0088375.g002

### Muscle Biopsies and Citrate Synthase Activity

Muscle biopsies were taken at baseline and five days after completing the training programme. The biopsies were obtained from the vastus lateralis with a sterile 5 mm diameter biopsy needle (Pelomi, Denmark) under local anaesthesia. All samples were snap-frozen in liquid nitrogen immediately after sampling. For citrate synthase (CS) maximal activity measurements, the muscle samples were homogenized in CellLytic buffer (Sigma-Aldrich, USA) at 6000 shakes/min for 2×8 seconds in a Precellys24 tissue homogenizer (Bertin, France). The homogenate was then centrifugated at 10000 g for 10 min at 4°C and supernatant tested for CS activity. The CS maximal activity was determined by the use of citrate synthase Assay Kit (CS 0720, Sigma-Aldrich). Absorbance at 412 nm was measured on a Fluostar Omega spectrometer (BMG Labtech, Germany). Specific activity was calculated by dividing measured activity on the muscle extract protein concentration. Protein concentrations were determined by the method of Bradford [17].

### Statistical Analysis

Samples size was estimated for VO<sub>2max</sub> as primary outcome measure using IBM SPSS SamplePower. The principal outcome variable VO<sub>2max</sub> were analysed between groups using a linear model with correction for baseline value (ANCOVA) [18], and within groups comparisons were done using paired samples T-test. Values are expressed as mean and standard deviation (SD). A two-tailed significance-level of p<0.05 was acknowledged as statistical significant. All analyses were done by PASW 17.0 (SPSS Inc, Chicago). At five occasions a subject was unavailable for testing. Because of the Gaussian behaviour of the data and the low number of missing values, these time points were estimated by an imputed value using Missing Value Analysis (PASW 17.0) to complete the dataset [19].

## Results

### Subject Characteristics

There were no differences between the groups with respect to age, body weight, height and body mass index (BMI) at baseline (Table 1). One person in each group withdrew from the study reporting tight calf muscles, and a total of 19 subjects completed the training- and detraining phases and were included in the analysis (Figure 1). All subjects that completed the study fulfilled > 95% compliance to the prescribed training programme.

### Adaptation of VO<sub>2max</sub>

There was no baseline difference in VO<sub>2max</sub> between the groups before the study started (Table 2). The MF group improved VO<sub>2max</sub> by 4.6% (p = 0.036) already after eight training sessions, whereas no significant improvement was seen in the HF group at the same time point (+2.3%, p = 0.128; between group difference p = 0.449). After finishing 16 of the 24 sessions, we found an

**Table 1.** Group Characteristics.

	MF group (n = 9)	HF group (n = 10)	p-value
Age (yrs)	23.1±2.3	23.7±2.1	= 0.560
Weight (kg)	70.2±16.8	67.7±8.9	= 0.684
Height (cm)	175.0±11.3	173.0±8.7	= 0.709
BMI (kg/m <sup>2</sup> )	22.7±3.1	22.6±2.3	= 0.938

doi:10.1371/journal.pone.0088375.t001

improvement in VO<sub>2max</sub> of 7.1% (p = 0.002) in the MF group compared no improvement (-0.5% vs. baseline, p<0.128) in the HF group (between group difference p = 0.036) (Figure 2 and Table 2). At post-test, i.e. four days after ending training, the MF group reached their highest achieved VO<sub>2max</sub> by 10.7% (p<0.001) above baseline, whereas no improvement (+3.0%, p = 0.126) was seen in the HF group (between group difference, p = 0.035). Twelve days into the detraining period (week 11 of the study) the HF group reached their highest VO<sub>2max</sub> with a value of 6.1% (p = 0.026) above baseline, compared to a VO<sub>2max</sub> of 7.9% (p<0.001) above baseline in the MF group at the same time point (group difference p = 0.609) (Figure 2). When considering the individual subjects' highest VO<sub>2max</sub> (Highest-VO<sub>2max</sub>), irrespective of which VO<sub>2max</sub>-test it was reached, the MF and HF groups improved their VO<sub>2max</sub> with 12.1% (p<0,001) and 7.5% (p = 0.010), respectively (between group difference, p = 0.319) (Table 2).

After 4 weeks of detraining (week 13 into the study, Figure 2 and Table 2) the VO<sub>2max</sub> in the MF group was still elevated compared to baseline (+2.9%, p = 0.029), but was reduced compared to their highest achieved time point (i.e. 4 day post exercise: p = 0.003). The VO<sub>2max</sub> in the HF group was after 4 weeks of detraining 4.2% above baseline (p = 0.018) and not reduced relative to the group's highest measurement (i.e. 12 d post exercise: p = 0.343) (Figure 2 and Table 2). After 6 weeks of detraining (week 15 into the study) VO<sub>2max</sub> was back to baseline level in both groups, i.e. +1.5% compared to baseline in the MF group, (p = 0.339) and -4.2% in the HF group (p = 0.064).

### Left Ventricular Adaptations

Stroke volume increased by 14.5% after the training period in the MF group (p = 0.028), while no change was observed in the HF group (p = 0.369) (Table 3). Left ventricular ejection fraction (EF) increased in the MF group (12.1%, p = 0.050), whereas it decreased by 11% (p<0.001) after HF training, but it is notable

**Table 2.** Values of VO<sub>2max</sub> expressed as means ± SD for both groups during the training and detraining periods.

Test points	MF group		HF group		Between gr.
	VO <sub>2max</sub>	p-value	VO <sub>2max</sub>	p-value	p-value
1 (pre-test)	51.5±5.5	-	52.2±7.0	-	= 0.290
2 (after 8 sessions)	<b>53.8±4.6</b>	<b>= 0.036</b>	53.4±7.9	= 0.128	= 0.449
3 (after 16 sessions)	<b>55.2±5.3</b>	<b>= 0.002</b>	51.9±8.5	= 0.829	<b>= 0.036</b>
4 (post-test, 24 sessions)	<b>56.9±6.1</b>	<b>&lt;0.001</b>	53.7±8.0	= 0.126	<b>= 0.035</b>
5 (12 d post exercise)	<b>55.6±5.0</b>	<b>&lt;0.001</b>	<b>55.4±9.8</b>	<b>= 0.026</b>	= 0.609
6 (4 wk post exercise)	<b>53.0±5.9</b>	<b>= 0.029</b>	<b>54.4±7.9</b>	<b>= 0.018</b>	= 0.798
7 (6 wk post exercise)	52.2±5.9	= 0.339	50.0±8.1	= 0.064	= 0.115
8 (post-detraining)	51.2±5.9	= 0.758	50.3±8.1	= 0.118	= 0.493
Highest-VO <sub>2max</sub>	<b>57.5±5.2</b>	<b>&lt;0.001</b>	<b>56.0±9.7</b>	<b>= 0.010</b>	= 0.319

Post-test refers to values at the first test after training, which was four days after last exercise session. Highest-VO<sub>2max</sub> refers to collected highest measured value of subjects within each group, independent of at which test point after training it occurred. VO<sub>2max</sub> is presented as ml · kg<sup>-1</sup> · min<sup>-1</sup>. Significant differences from baseline within groups and differences between groups are typed in bold. Between group differences are post-test values.

doi:10.1371/journal.pone.0088375.t002

that EF was 15% higher in the HF group compared to MF group at baseline (between group difference,  $p=0.050$ ). End diastolic volume (EDV) increased only in the HF group, by 10.1% ( $p=0.050$ ).  $S'$  tended to increase in the MF group (7.8%,  $p=0.056$ ), but no change was observed in the HF group (between group differences:  $p=0.490$ ). E, A and  $e'$  did not change in any group after the training period.

During the detraining period SV and EF returned to baseline values in the MF group. In the HF group SV increased by 10.0% ( $p=0.031$ ) during the detraining, and EF and  $S'$  returned to baseline values.

### Lung Function

Flow volume spirometry values did not change at any point of measurement in either group. TLCO was reduced by 11.3% ( $p=0.004$ ) in the HF group after the training period, while the MF group increased diffusion capacity by 6.0% ( $p=0.041$ ) (Table 4). Haemoglobin levels did not differ during the study period, and thus TLCO was not corrected for haemoglobin levels.

### Endothelial Function

Flow mediated dilation was unchanged from before to after training both within the MF group ( $7.4\pm 3.6$  vs.  $7.5\pm 2.5\%$  ( $p=0.952$ )) and within the HF group ( $7.5\pm 2.0$  vs.  $7.6\pm 2.8\%$  ( $p=0.910$ )) (between group difference  $p=0.943$ ) (Table 4).

### Citrate Synthase Activity

The vastus lateralis citrate synthase maximal activity relative to the muscle extract protein concentration was increased by 39% in the MF group (from  $0.38\pm 0.10$  to  $0.52\pm 0.10$   $\mu\text{mol} \cdot \text{ml}^{-1} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$  protein,  $p<0.001$ ) (Table 4). We also found a 25% increase in activity in the HF group (from  $0.30\pm 0.12$  to  $0.37\pm 0.11$   $\mu\text{mol} \cdot \text{ml}^{-1} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$  protein), but this improvement did not reach statistical significance ( $p=0.185$ ; between group difference  $p=0.009$ ).

### Discussion

The main finding of this study was that both HF and MF training of high-intensity aerobic exercise improves  $\text{VO}_{2\text{max}}$ . We found however no improvements in  $\text{VO}_{2\text{max}}$  immediately after

ending the HF training programme. In addition, we found a reduction in lung diffusion capacity and indications of a reduction in cardiac function in HF immediately after training. This indicates a possibility of fatigue when exposed to such a high exercise dose. However, during the detraining period there was a delayed increase in  $\text{VO}_{2\text{max}}$  in the HF group demonstrating the necessity of sufficient rest to adapt from such a heavy exercise training programme. The MF training programme, in contrast, had a positive effect for improving the aerobic capacity in a progressive manner throughout the training period.

### Maximal Oxygen Uptake

Previous studies have demonstrated that  $\text{VO}_{2\text{max}}$  improves after HF aerobic training [20,21], suggesting a possibility to improve  $\text{VO}_{2\text{max}}$  in reduced time compared to more moderate training regimes. In line with this, a recent study by Breil et al. [8] involving Swiss alpine skiers, showed that it is possible to boost the aerobic capacity by means of a 6% increase of  $\text{VO}_{2\text{max}}$  using a high-intensity interval training “shock” of 15 sessions in 11 days. In our study we found a quite different progression of the adaptation between MF- and HF-training, and the highest  $\text{VO}_{2\text{max}}$ -values occurred at different time points. In the MF group the adaptation to physical training progressed in a regular manner.  $\text{VO}_{2\text{max}}$  increased progressively in the MF group during the training period, and reached highest value at post-test, four days after ending training period (+10.7%,  $p<0.001$ ). Contrary, in the HF group there was no change in  $\text{VO}_{2\text{max}}$  during the training period. In fact, we measured a small, but not significant reduction at the  $\text{VO}_{2\text{max}}$ -test performed at the 16<sup>th</sup> training session, 14 days into the training regime. Furthermore, the HF group showed no improvements four days after ending 24 sessions of training (+2.9%,  $p=0.126$ ), and improved their  $\text{VO}_{2\text{max}}$  first after another week of detraining (+6.1%,  $p=0.026$ ), i.e. 12 days after finishing training. Interestingly, despite training for only 3 weeks the HF group still required 5 weeks to reach their highest measured  $\text{VO}_{2\text{max}}$  (+6.1% from baseline) whereas the MF group reached an improvement of 7.1% immediately after completing their 6 weeks of moderate training.

Our results show that the highly intensified training load experienced by the HF group was too severe to progressively adapt compared to the MF training programme. It is assumed that

**Table 3.** Parameters of heart function for both groups before and after training and after detraining.

	MF group					HF group				
	Pre-test	Post-test	p-value	Detraining	p-value	Pre-test	Post-test	p-value	Detraining	p-value
<b>HR<sub>rest</sub></b> , b/min	58±6	58±9	=0.997	59±9	=0.901	65±9	57±8	=0.034	66±12	=0.954
<b>BP<sub>sys</sub></b> , mmHg	117.0±10.0	118.0±11.0	=0.943	108.0±9.0	=0.025	118.5±10.5	123.0±9.0	=0.343	121.5±16	=0.561
<b>BP<sub>diar</sub></b> , mmHg	74.0±6.0	74.0±7.0	=0.992	68.0±3.0	=0.074	74.0±8.5	74.0±5.0	=0.784	71.0±9.5	=0.454
<b>EDV</b> , ml	120.0±28.0	129.0±40.0	=0.176	118.6±23.0	=0.408	119.5±20.0	131.5±25.0	=0.034	131.0±20.0	=0.024
<b>SV</b> , ml	77.0±21.0	88.0±18.0	=0.028	87.0±21.0	=0.192	85.0±14.1	89.0±18.5	=0.369	93.5±11.0	=0.031
<b>S'</b> , cm/s	8.95±8.1	9.65±7.5	=0.056	9.05±10.5	=0.096	9.50±16.1	9.60±14.3	=0.813	9.70±14.6	=0.507
<b>EF</b> , %	58.0±5.8	65.0±4.3	=0.050*	63.5±5.9	=0.380	66.5±5.1	59.0±2.7	<0.001	66.0±5.2	=0.805
<b>E/A</b>	2.4±0.7	2.7±0.9	=0.239	2.4±0.7	=0.819	2.3±0.8	2.8±0.6	=0.070	2.3±0.7	=0.790
<b>e'</b>	15.9±2.2	16.1±2.4	=0.741	16.0±1.8	=0.524	15.6±2.6	15.7±1.9	=0.862	15.7±2.1	=0.734

Values are expressed as means ± SD. HR<sub>rest</sub>: resting heart rate, BP<sub>sys</sub>: systolic blood pressure, BP<sub>diar</sub>: diastolic blood pressure, EDV: end diastolic volume, SV: stroke volume, S': peak annulus velocity in systole, e': early diastole, and A': late diastole were measured as average of the four measures. EF: ejection fraction. P-values are within-groups differences from pre- to post-test, and between group differences for post-test values.

\* Significant differences between groups ( $p<0.05$ ).

doi:10.1371/journal.pone.0088375.t003

**Table 4.** Physiological variables for both groups before and after the training period.

	MF group		p-value	HF group		p-value	Between gr. p-value
	Pre-test	Post-test		Pre-test	Post-test		
FMD, %	7.4±3.6	7.5±2.5	= 0.952	7.5±2.0	7.6±2.8	= 0.910	= 0.948
TLCO, conductance units	9.36±1.16	9.92±1.48	= 0.041	10.61±2.14	9.40±1.79	= 0.004	= 0.094
CS, $\mu$ mole/ml/min/mg protein	0.38±0.10	0.52±0.09	<0.001	0.30±0.12	0.37±0.12	= 0.185	= 0.009

Values are expressed as means  $\pm$  SD. FMD; Brachial artery flow mediated dilatation, CS; citrate synthase activity, TLCO; transfer factor of the lung for carbon monoxide. Between group differences are post-test values. P-values are within-groups differences from pre- to post-test, and between group differences for post-test values. doi:10.1371/journal.pone.0088375.t004

recovery processes continue through homeostatic restoration of the body after training until a compensation effect may occur [22]. The most common way to adapt and improve physical fitness after training is to recover sufficiently and then perform the next exercise session at the peak of the compensation phase [23]. However, It has been described that athletes may experience acute feelings of fatigue and decrease in performance following periods of intensified training [24]. Intensified training can thus result in a temporarily decline in performance, but when sufficient periods of recovery are provided, a supercompensation may occur with enhanced performance compared to baseline levels [24]. As it is possible to recover within a 2-week period [25], it may be argued that this is a relatively normal condition and thus a normal part of a strenuous training process. The lack of improvement of  $VO_{2max}$  in the HF group during the training period, along with the compensation effect in the detraining phase, suggests that too severe training load was induced, as opposed to the progressive improvement of  $VO_{2max}$  in the MF group suggesting sufficient rest between the exercise sessions. However, it is still possible that more well-trained athletes could have acquired greater adaptation to HF training compared with our moderately trained students. There was however only one drop-out in each group, despite the higher physical demands in the HF group. The high compliance rate might be due to the young healthy population and a strong will to complete the training programme.

After 6 weeks of detraining, that started after the last exercise session,  $VO_{2max}$  returned to baseline values both in the MF and HF group. Mujika and Padilla [26] found that recently trained subjects most often reverse their gained  $VO_{2max}$  when detraining extends 4 weeks [27], whereas Pedersen et al. [28] found that the inactivity period simply reverses the training effect in the same manner as it emerged. Coyle et al. found that the decline in  $VO_{2max}$  stabilized after 8 weeks of detraining [29]. Although  $VO_{2max}$  in both groups in our study were still elevated compared to baseline  $VO_{2max}$  at 4 weeks into the de-training period, our findings suggest an earlier reduction of  $VO_{2max}$  relative to highest measured value in the MF group, i.e. after 4 weeks compared to 6 weeks in the HF group. The reason for this may simply correspond to the latency of their highest  $VO_{2max}$  value in the HF group, which occurred 2 weeks after training cessation. The gained aerobic capacity in both groups thus declined in a similar pattern from the highest reached value, being back at baseline 4 weeks after their highest measurement.

### Cardiovascular Response

We observed that stroke volume increased significantly in the MF group in response to the interval training in line with other studies [2,5,30]. In the HF group we found an increased end diastolic volume. In the HF group EF was reduced, suggesting a depressed left ventricular adaptation after the training period. The

reduction in the systolic function is in concordance with another study that revealed a negative myocardial effect immediately after a strenuous exercise programme [12]. It should be emphasized that heart rate was reduced in the HF group after the exercise period, which may have affected the cardiac measurements. The cardiac measurements were secondary endpoints and the study is thus too small to draw firm conclusion. As expected, the diastolic measurements at rest did not improve, confirming existing literature that young healthy sedentary subjects on beforehand have a normal and optimal diastolic function [31].

During detraining in the MF group we observed that the stroke volume and the EF returned to baseline values. This indicates loss of the training induced adaptations caused by inactivity. The groups however differed in their adaptations during detraining. Stroke volume increased during detraining amongst the HF group and this was attributable to the increased end diastolic volume. EF was subsequently normalized. This indicates that recovery is crucial to achieve cardiac improvements after a strenuous physical activity programme. However, although the HF group increased stroke volume during detraining, the inactivity period returned  $VO_{2max}$  to baseline values.

It has previously been suggested that intense exercise may induce stress that impairs the systemic endothelial function [32]. We found however no signs of endothelial dysfunction as both groups showed the same FMD of the brachial artery both before and after training.

### Lung Function

The MF group increased pulmonary diffusion capacity after the training period. Diffusion capacity may have increased in relation to the increased stroke volume seen in the MF group.

### Peripheral Adaptations

The mitochondrial citrate synthase maximal activity improved more in the MF group compared to the HF group after the training programmes. Our results are in accordance with previous high-intensity exercise training studies showing that maximal activity of the citrate synthase is normally upregulated by 15–35% after strenuous training [33]. In line with our results, a recent study concluded that in skeletal muscle of trained individuals, the maximal activity of citrate synthase were enhanced to a greater extent by having a day of rest between the exercise sessions compared with training daily for 3 weeks [34].

### Study Strengths and Limitations

This study is small with few subjects in each group. However, the high number of test points throughout the study with a steady incline and decline of  $VO_{2max}$  in the MF group, is indicating that changes not just being measurement variations. Also, the  $VO_{2max}$ -tests consistently showed plateaus of the  $O_2$ -curve and high RER-

values above 1.10, suggesting maximal effort during all tests. As it is difficult to predict when each subject achieves their highest  $\text{VO}_{2\text{max}}$  value after a training programme, an even more frequent test programme could have revealed even higher maximum values. More frequent testing could on the other hand elicit an unwanted training effect in the detraining period. Another aspect would have been to elucidate which mechanisms were causing the signs of fatigue during and after HF training. However, this study was not designed to examine these underlying mechanisms. The study was originally designed also to investigate retraining after the detraining period, but due to the strain of the participants, this part of the study was not implemented.

## Conclusions

It has previously been shown that it is possible to boost  $\text{VO}_{2\text{max}}$  by a short and intensified high-intensity interval training period. In our study we found a different progression of the adaptation between MF- and HF-training, and the highest  $\text{VO}_{2\text{max}}$ -values occurred at different time points. The adaptation following a HF programme of high-intensity exercise was delayed compared to training with MF. The HF programme may induce an initially

fatigue which may limit the cardiopulmonary function whereas the latter programme follows a more progressive adaptation.

## Supporting Information

**Protocol S1 Trial protocol.**  
(DOC)

**Checklist S1 CONSORT checklist.**  
(DOC)

## Acknowledgments

We would like to thank Cecilie Horvei for conducting the lung function measurements.

## Author Contributions

Conceived and designed the experiments: HH PKS HEM EB AET SS ES UW CBI ØR. Performed the experiments: HH PKS HEM EB AET CBV ØR. Analyzed the data: HH PKS HEM EB SS ES UW CBV ØR. Contributed reagents/materials/analysis tools: HH PKS HEM EB CBV ØR. Wrote the paper: HH PKS HEM UW ØR.

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