Moderate vs. high exercise intensity: Differential effects on aerobic fitness, cardiomyocyte contractility, and endothelial function

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Abstract

Objective: Current guidelines are controversial regarding exercise intensity in cardiovascular prevention and rehabilitation. Although high-intensity training induces larger increases in fitness and maximal oxygen uptake (VO_{2max}), moderate intensity is often recommended as equally effective. Controlled preclinical studies and randomized clinical trials are required to determine whether regular exercise at moderate versus high intensity is more beneficial. We therefore assessed relative effectiveness of 10-week HIGH versus moderate (MOD) exercise intensity on integrative and cellular functions.
Methods: Sprague–Dawley rats performed treadmill running intervals at either 85%–90% (HIGH) or 65%–70% (MOD) of VO_{2max} 1 h per day, 5 days per week. Weekly VO_{2max}-testing adjusted exercise intensity.
Results: HIGH and MOD increased VO_{2max} by 71% and 28%, respectively. This was paralleled by intensity-dependent cardiomyocyte hypertrophy, 14% and 5% in HIGH and MOD, respectively. Cardiomyocyte function (fractional shortening) increased by 45% and 23%, contraction rate decreased by 43% and 39%, and relaxation rate decreased by 20% and 10%, in HIGH and MOD, respectively. Ca^{2+} transient time-courses paralleled contraction/relaxation, whereas Ca^{2+} sensitivity increased 40% and 30% in HIGH and MOD, respectively. Carotid artery endothelial function improved similarly with both intensities. EC_{50} for acetylcholine-induced relaxation decreased 4.3-fold in HIGH ($p < 0.05$) and 2.8-fold in MOD ($p < 0.20$) as compared to sedentary; difference HIGH versus MOD 1.5-fold ($p = 0.72$). Multiple regression identified rate of systolic Ca^{2+} increase and diastolic myocyte relengthening as main variables associated with VO_{2max}. Cell hypertrophy, contractility and vasorelaxation also correlated significantly with VO_{2max}.
Conclusions: The present study demonstrates that cardiovascular adaptations to training are intensity-dependent. A close correlation between VO_{2max}, cardiomyocyte dimensions and contractile capacity suggests significantly higher benefit with high intensity, whereas endothelial function appears equivalent at moderate levels. Thus, exercise intensity emerges as an important variable in future preclinical and clinical investigations.
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Keywords: Maximal oxygen uptake; Cardiomyocyte; Exercise training; Endothelium; Calcium; Contractile function

1. Introduction

Recent clinical and epidemiological studies suggest that beneficial effects of regular physical exercise may depend on intensity or amount of work performed during training [1–6]. This is consistent with the observation that aerobic exercise capacity measured as maximal oxygen uptake (VO_{2max}) or metabolic equivalents is a major predictor of all-cause mortality both in normal subjects and cardiovascular disease [7–9]. In contrast, current recommendations for prevention and rehabilitation range 40%–90% of VO_{2max} [10,11], probably because of controversies regarding the biological effects and clini-

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cal feasibility of moderate versus high intensity training [12].

Both clinical and experimental studies have linked improved aerobic fitness, cardiovascular function and all-cause mortality to vascular endothelial [13–15] and cardiac function [16–19]. Cellular mechanisms include physiological cardiomyocyte hypertrophy, reduced remodelling, increased contractility [19–22] and enhanced Ca^{2+} handling [19,23,24], which all translate into better pump function. Increased arterial dilation improves myocardial oxygen supply [15], and may indicate additional endothelium-dependent functions that prevent ischemic events. A recent study from our laboratory showed that changes in aerobic fitness were closely associated with several aspects of cardiomyocyte contractile capacity and Ca^{2+} handling during the course of exercise training (2–13 weeks) and detraining (2–4 weeks), whereas endothelium-dependent arterial relaxation was more loosely correlated [25]. Based on these observations and the fact that high versus moderate intensity is more favourable for aerobic capacity in humans [5], the working hypothesis of the present study was that increased VO_{2max} in response to exercise parallels improvement of cardiomyocyte contractile capacity over a wide range of intensities, while endothelial function may have different dynamics.

2. Methods

2.1. Study design and animals

A total of 24 female adult Sprague–Dawley rats (Møllegaards Breeding Centre Ltd., Lille Skensved, Denmark), age 80–90 days at start of training were randomized into three groups, high (HIGH) and moderate intensity (MOD), and sedentary control. When VO_{2max} remained unchanged for 3 consecutive weeks, which occurred after 10 weeks, the rats were sacrificed under full etherisation. The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The Norwegian Council for Animal Research approved experimental protocols.

2.2. Maximal oxygen uptake and training

Before and after the experimental period, and at the start of every training week to determine intensity, VO_{2max} was measured as previously described during 25° (47%) treadmill-running [18,19]. Rats warmed up for 20 min at 50%–60% of VO_{2max}, whereupon treadmill velocity was increased by 0.03 m s^{-1} every 2 min until they were unable to, or refused to, run further. The criterion for reached VO_{2max} was a levelling-off of oxygen uptake (VO_2) despite increased workload. Training rats performed interval-running 1 h day^{-1}, 5 days·week^{-1} on the 25° treadmill. After 10 min warming up at 50%–60% of VO_{2max}, rats ran

5 intervals, alternating between 8 min at 85%–90% (HIGH) or 65%–70% (MOD) of VO_{2max} and 2 min at 50%–60% in the high and moderate intensity groups, respectively. Intensity was adjusted weekly, and all rats randomized to training completed the exercise program. Sedentary rats maintained treadmill-running skills for 15 min on flat treadmills at 0.15 m s^{-1} twice weekly. This activity did not yield any training response; the intensity corresponded to 44.5±7.5% and 47.5±7.5% of VO_{2max} before and after the regimen, respectively.

2.3. Cardiomyocyte isolation and measurements

Left ventricular cardiomyocytes were isolated as previously described [19,22,25], with a modified Krebs–Henseleit Ca^{2+}-free buffer with collagenase-2, and $CaCl_2$ 1.2 mM added stepwise. Cardiac ventricles were weighed after perfusion, which induced a substantial increase in tissue water content. Cardiomyocytes rested 1–3 h on laminin-coated coverslips in HEPES buffer 37 °C, before 20-min loading with 2 μM Fura-2/AM (Molecular Probes, Eugene, OR) and 0.3% dimethyl sulfoxide (Sigma Chemical, St. Louis, MO). Cells were placed in a cell chamber (37 °C) on an inverted microscope (Diaphot-TMD, Nikon, Tokyo, Japan) and stimulated electrically by bipolar pulses (5 ms duration). A 500 Hz rotating mirror alternated ultraviolet excitation light through band-pass filters of 340 and 380 nm, while 510 nm fluorescence emission was counted with a photomultiplier tube (D-104, Photon Technology International, Lawrenceville, NJ), and expressed as the ratio of the 2 excitation wavelengths. Intracellular Ca^{2+} transients and their time-courses were measured, together with video edge-detection (Model 104, Crescent Electronics, Sandy, UT) of cell shortening and relaxation with time-courses. Ten stable, consecutive contractions at each stimulation frequency (2, 5, 7, 10 Hz), after a steady state was reached (usually within 10–30 s), were studied in 5–10 cells per animal. From every animal, 150 cells not introduced to Fura-2/AM-DMSO and without obvious cellular damage were measured for length and midpoint width. Cell volume was estimated as cell length·width·0.00759, as established by 2D light and 3D confocal microscopy [26]. Cell yield (>2·10^6, >70% rod-shaped and viable) and post-pacing cell deaths were rare (<5–10%) and occurred with similar frequencies in all groups.

2.4. Echocardiography

Echocardiography was performed the last week of training, after sedation with 40 mg kg^{-1} ketamine hydrochloride and 8 mg kg^{-1} xylazine intraperitoneally, with a 10 MHz linear array probe and system FiVe ultrasound scanner (GE Vingmed Ultrasound, Horten, Norway). Heart rate, fractional shortening and left ventricular dimensions were calculated as the mean of 5 consecutive cardiac cycles in 2-dimensional M-mode long-axis recordings. Mitral inflow
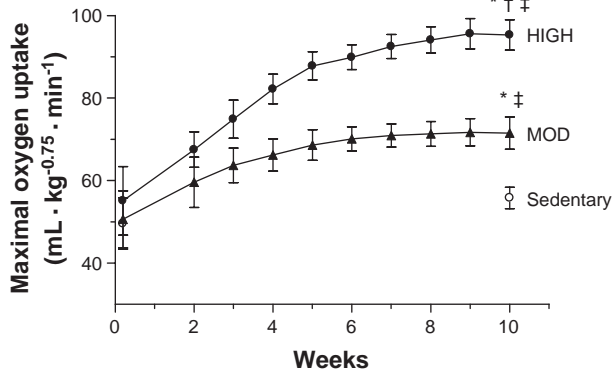
Fig. 1. Time course of maximal oxygen uptake during 10 weeks of HIGH or moderate (MOD) intensity treadmill running, and sedentary controls. Data are means ± SD. HIGH/MOD vs. sedentary: $*p < 0.01$. HIGH vs. MOD: $†p < 0.01$. Endpoint vs. baseline: $‡p < 0.01$.

deceleration time, peak velocity of early and late component of mitral inflow, and isovolumetric relaxation time were calculated as the mean of 5 consecutive cardiac cycles of pulsed-wave Doppler spectra recordings.

2.5. Endothelial function

L-shaped holders were inserted into the lumen of 2–4 mm segments of the common carotid arteries; 1 holder connected to a force-displacement transducer and the other to a micrometer, in organ baths containing Krebs buffer and indomethacin [25,27]. After gradually increasing tension to 1000 mg, and exposure to $6 \cdot 10^{-2}$ M K^+, $3 \cdot 10^{-7}$ M phenylephrine and 10^{-4} M acetylcholine to ensure reactivity, segments were equilibrated 30 min before experiments began. Four segments per animal were pre-contracted with phenylephrine ($3 \cdot 10^{-7}$ M) and relaxed with cumulative doses of acetylcholine (2 segments) and Na^+ nitroprusside (1 segment), whereas 1 segment was pre-treated with 10^{-4} M N_ω-nitro-L-arginine methyl esther (L-NAME) before exposure to acetylcholine. To assess arterial sensitivity to acetylcholine, we estimated the agonist concentration for half relaxation (EC_{50}) of acetylcholine-induced relaxation as previously described [28]. However, as the accumulated dose-responses did not completely reach maximal states, we first estimated the individual levelling-off with conventional, variable slope curve-fitting methods (GraphPad Software Inc., San Diego, CA), whereupon individual EC_{50}-values were obtained.

2.6. Allometric scaling

Although training regimens alter cardiac muscle weights and VO_{2max}, differences may also be due to a growing body mass. Thus, when lean body mass is unavailable, allometric scaling should be applied [29]. Ventricular mass should hence be expressed in relation to body weight raised to the power of 0.78 [30], and VO_{2max} with the scaling exponent 0.75 [31].

2.7. Statistics

Data are expressed as mean ± SD, with significance level $p < 0.05$. The Friedman test and appropriate procedures for multiple comparisons were used to determine changes in VO_{2max} throughout the experimental period. The Kruskal–Wallis with post-hoc test and one-way ANOVA with Scheffe post-hoc test (not presented as different approaches yielded similar results) evaluated unrelated observations between groups, whereas repeated measures ANOVA with Scheffe post-hoc analysis determined group differences between repeatedly measured variables. Relationships were deter-
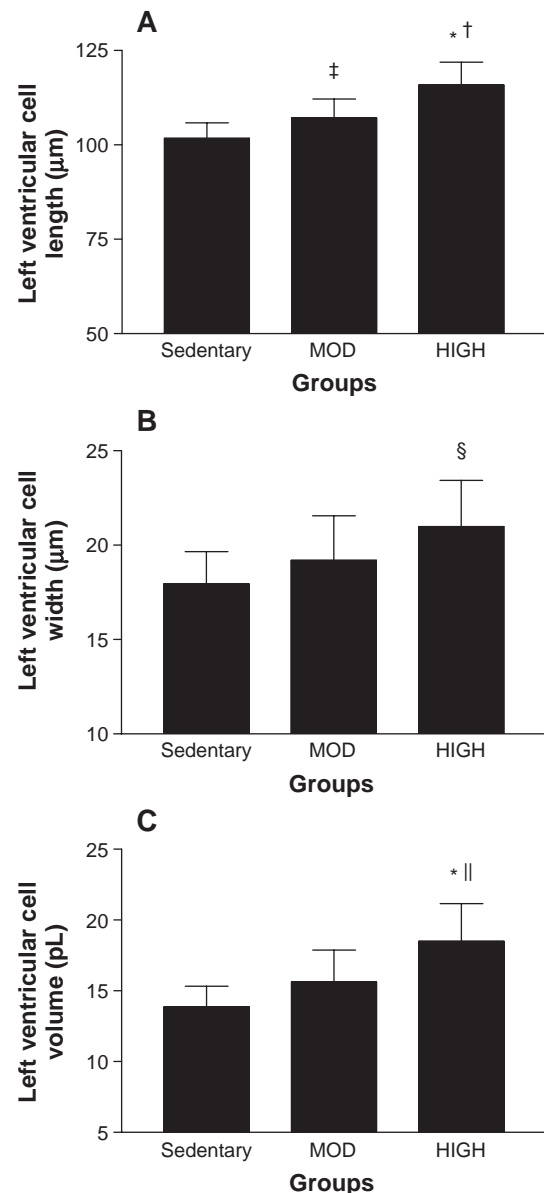

Fig. 2. Cardiomyocyte length (panel A), width (panel B) and estimated volume (panel C) in HIGH and moderate intensity (MOD) trained and sedentary rats, in 3600 cells, 150 per rat. Data are mean ± SD. HIGH vs. sedentary: $*p < 0.01$, $§p < 0.05$. HIGH vs. MOD: $†p < 0.01$, $\|p < 0.05$. MOD vs. sedentary: $‡p < 0.05$.

mined with Pearson's correlation coefficient and univariate, forward and backward multiple linear regression. Maximal oxygen uptake was modelled with explanatory variables cardiomyocyte dimensions and volume, shortening, contraction and relaxation time-courses, Ca^{2+} transient time-courses and Ca^{2+} sensitivity, and arterial responsiveness to acetylcholine (EC_{50}). Exclusion criterion was $p > 0.05$.

3. Results

3.1. Aerobic capacity

Maximal oxygen uptake increased by 71% in HIGH and 28% in MOD (Fig. 1), whereas maximal aerobic running velocity increased by 112% (0.25 ± 0.01 to 0.53 ± 0.03 m
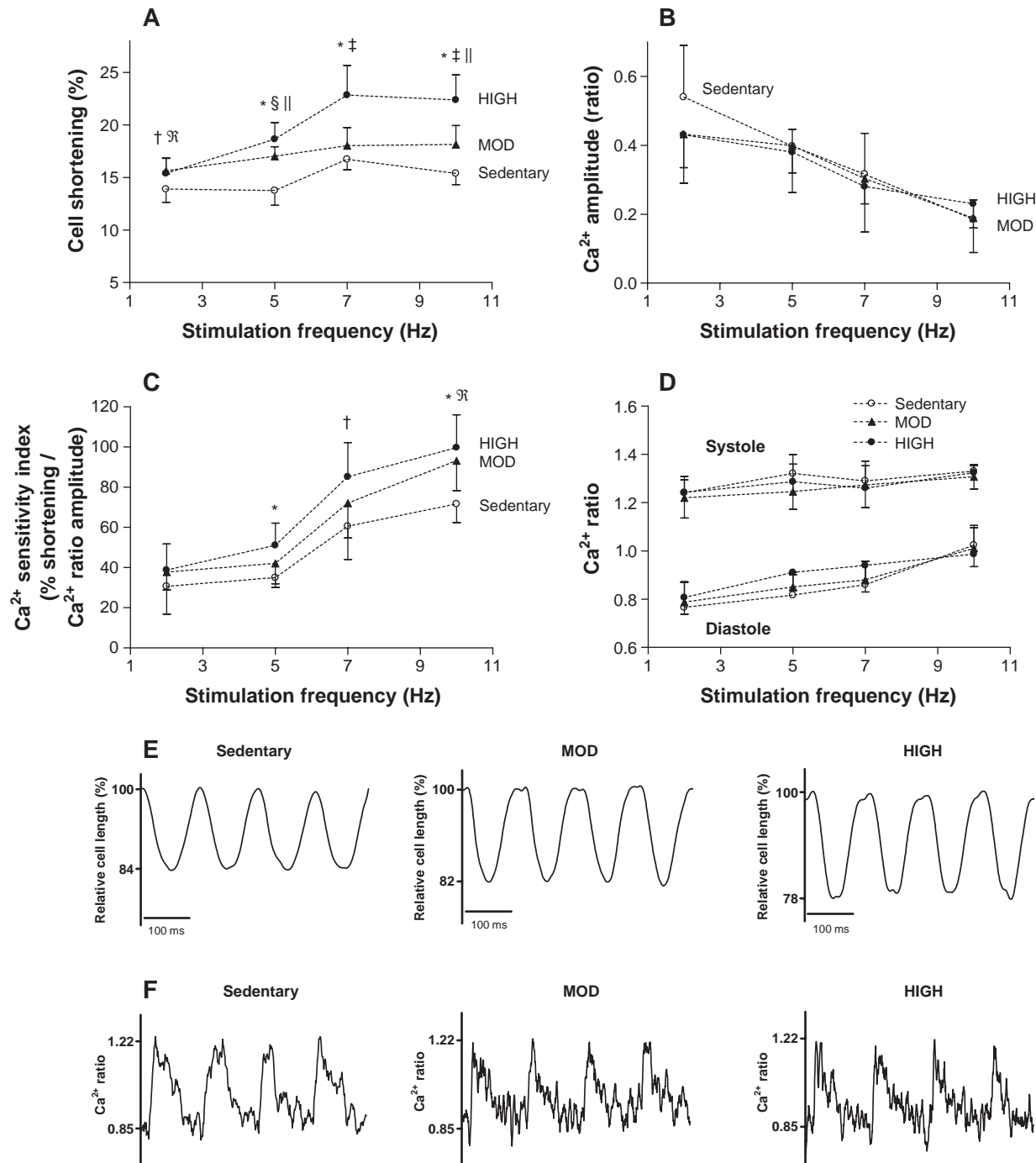

Fig. 3. Cardiomyocyte shortening (panel A), Ca^{2+} ratio amplitude (panel B), Ca^{2+} ratio sensitivity index (panel C), and diastolic and systolic Ca^{2+} ratios (panel D) in HIGH and moderate intensity (MOD) trained and sedentary rats, in 5–10 cells per rat. Data are mean±SD. HIGH vs. sedentary: *$p < 0.01$, †$p < 0.05$. HIGH vs. MOD: ‡$p < 0.01$, §$p < 0.05$. MOD vs. sedentary: ‖$p < 0.01$, $p < 0.05$. Typical cardiomyocyte shortening (panel E) and Ca^{2+} transient (panel F) traces at 7 Hz stimulation from each group are displayed.

s^{-1}, $p < 0.01$) and 38% (0.24 ± 0.01 to 0.33 ± 0.03 m s^{-1}, $p < 0.01$) in HIGH and MOD, respectively. Sedentary rats plateaued at 0.25 ± 0.02 m s^{-1} both before and after the experimental period.

3.2. Cardiomyocyte hypertrophy and contractility

Exercise induced intensity-dependent cardiomyocyte hypertrophy. Isolated left ventricular cardiomyocytes were 14% longer in HIGH, versus 5% in MOD. Width and volume increased significantly in HIGH, whereas trends

occurred in MOD (Fig. 2). Myocyte contractile function during electrical stimulation at physiological frequencies (7–10 Hz) improved about twice as much in HIGH as in MOD. Cell fractional shortening increased by 45% in HIGH compared to sedentary, and by 26% when compared to MOD, but only 23% in MOD (Fig. 3). Conditioning induced parallel reductions in time-course of cell shortening and Ca^{2+} transient, both during contraction and relaxation. HIGH decreased time to 50% and peak contraction by 35% and 43%, respectively (Fig. 4). MOD decreased time to peak contraction (39%), and a ~20% difference occurred
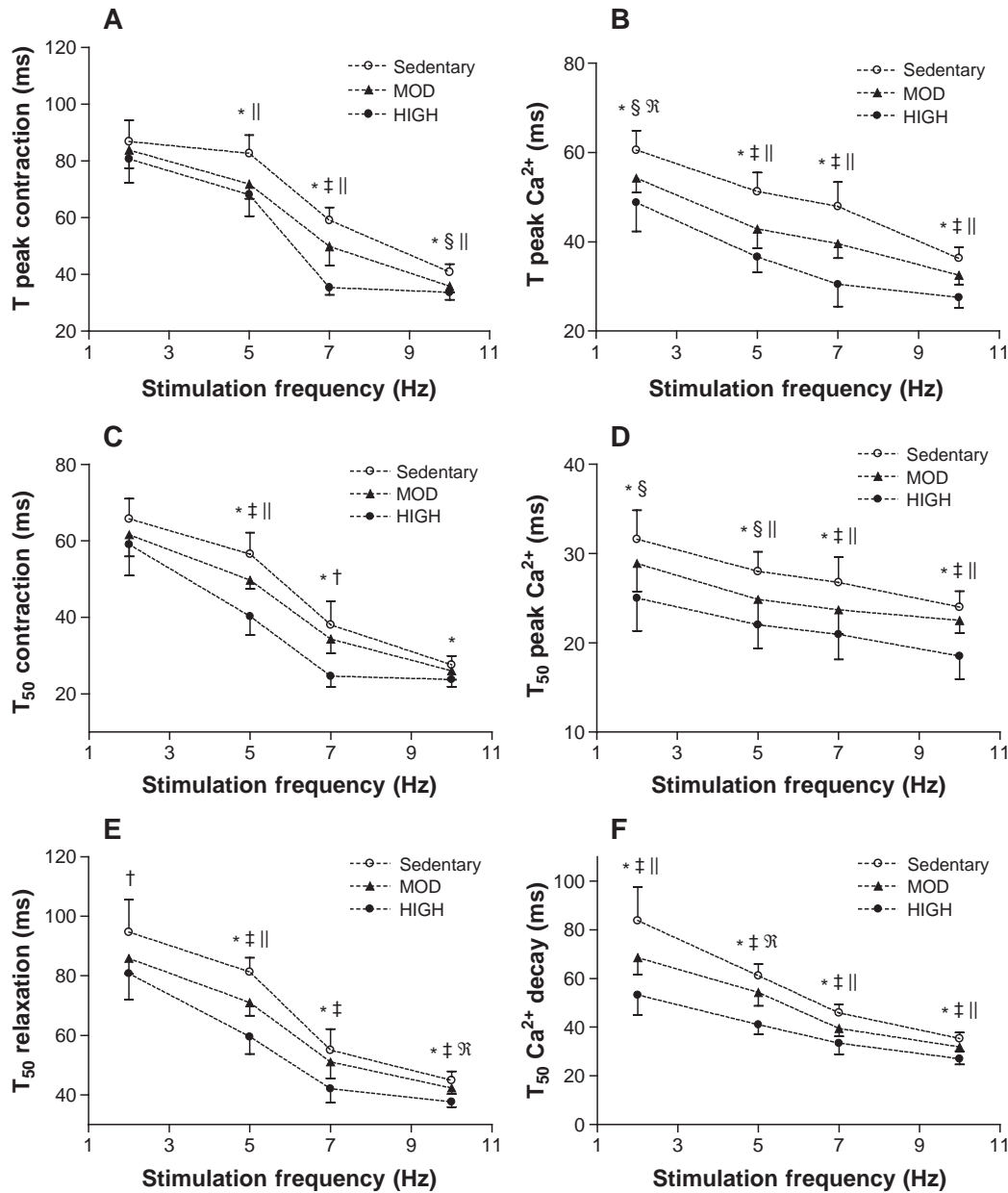

Fig. 4. Time-course of contraction/relaxation and Ca^{2+}-transient in HIGH and moderate intensity (MOD) trained and sedentary rats, in 5–10 cells per rat. Panels A and B show time to peak contraction and peak Ca^{2+} ratio, panels C and D show half-time to peak contraction and peak Ca^{2+} ratio, and panels E and F show half-time to relaxation and Ca^{2+} ratio decay, respectively. Data are mean $\pm$ SD. HIGH vs. sedentary: $*p < 0.01$, $\dagger p < 0.05$. HIGH vs. MOD: $\ddagger p < 0.01$, $\S p < 0.05$. MOD vs. sedentary: $\| p < 0.01$, $p < 0.05$.

between HIGH and MOD. The training response for diastolic function, measured as cell re-lengthening after peak contraction, was similar, with HIGH decreasing ~20% and MOD ~10%, with $p < 0.01$ for difference between them (Fig. 4). Time to peak Ca^{2+} and diastolic decay correlated closely with contraction–relaxation time-courses, with fastest response in HIGH cells and slowest in sedentary (Fig. 4). Systolic and diastolic Fura-2 Ca^{2+} ratios were unaffected by training, indicating increased myofilament responsiveness to Ca^{2+}. The shortening/Ca^{2+}-amplitude index was ~40% higher in HIGH and ~30% in MOD versus sedentary (Fig. 3).

3.3. Arterial endothelial function

Acetylcholine-mediated endothelium-dependent artery relaxation increased with training, but a difference between HIGH and MOD was barely indicated (Fig. 5). As artery relaxation did not plateau upon accumulating doses of acetylcholine, we assessed arterial sensitivity to acetylcholine by first estimating maximal relaxation and then calculating agonist concentration for half relaxation (EC_{50}) of each animal. Reduced EC_{50} demonstrated improved vessel sensitivity to acetylcholine, expressed as a log-scale (HIGH: -6.99 ± 0.57; MOD: -6.81 ± 0.38; Sedentary: -6.36 ± 0.45) representing 4.3-fold difference for HIGH ($p < 0.05$) and 2.8-fold for LOW ($p = 0.20$) compared to sedentary, and similar sensitivity (1.5-fold difference, $p = 0.72$) for HIGH versus MOD. Thus, improvement of endothelium-mediated vasodilatation seems close to plateauing with training at moderate exercise intensity.

3.4. Correlation and regression analysis linking VO_{2max} to cellular adaptations

In univariate analysis, VO_{2max} correlated strongly with cardiomyocyte dimensions and volume, fractional shortening and contraction–relaxation time-courses, Ca^{2+} ratio transient time-courses, Ca^{2+} sensitivity index, and artery acetylcholine-mediated relaxation (Fig. 6). Univariate correlation also demonstrated close inter-dependence between intrinsic cardiomyocyte features, whereas endothelial function did not correlate significantly with cardiomyocyte features (data not shown). This is expected as related intrinsic variables of myocyte hypertrophy, contractility and Ca^{2+} handling are internally linked, whereas intrinsic vasoreactivity seems independent of cardiomyocyte features. Forward (not shown) and backward multiple regression identified the main cellular factors determining VO_{2max} and its response to different training regimens. Half-times to peak Ca^{2+} and myocyte relaxation emerged as the main determinants for VO_{2max}, with unstandardized coefficients b $-2218.68 \pm SE$ 375.21 ($p < 0.01$), and $-580.25 \pm SE$ 217.47 ($p < 0.02$), respectively; residual SD $= 8.05$, adjusted $R^2 = 0.75$, while cell volume had a clear trend ($p = 0.13$).

3.5. Cardiac weights and echocardiography

As expected from the clear effects on cell size, intraventricular septum and posterior wall thickness showed either statistically significant or strong trends for exercise-induced left ventricle hypertrophy. A weaker trend to diastolic left ventricle diameter with unchanged systolic diameter indicated higher chamber size (Table 1). These observations are consistent with lower sensitivity of echocardiography due to random variation, which requires larger groups to detect biologically important changes [32]. Parallel discrepancy between echocardiography and cellular measurements has previously been reported [20]. In contrast, trends for cardiac hypertrophy, as judged by left and right ventricle weights after collagenase perfusion were rather weak, probably because of massive swelling that seemed to vary substantially among hearts (Table 2).

4. Discussion

The present study demonstrates that effectiveness of regular exercise regarding cellular functions associated with aerobic capacity depends on the intensity of the training program. Our experiments indicate that cardiovascular effects related to VO_{2max}, cardiomyocyte contractility and Ca^{2+} handling require high exercise intensity for full
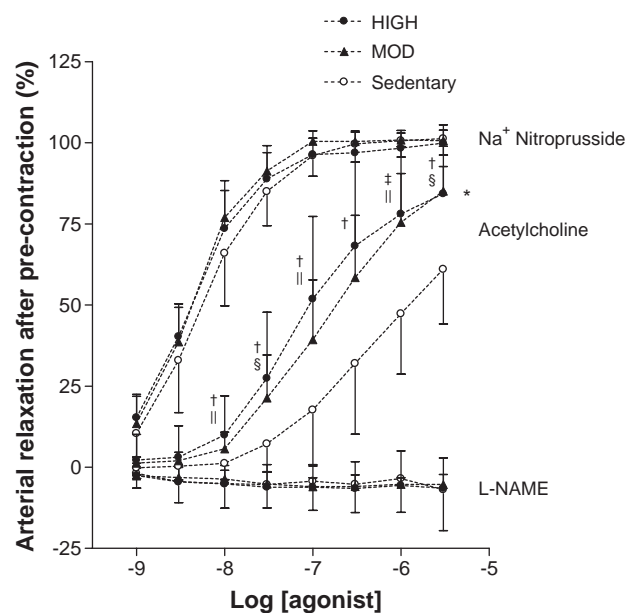

Fig. 5. Phenylephrine-precontracted carotid artery response to accumulating acetylcholine, acetylcholine+L-NAME, or Na$^+$ nitroprusside, with presence of indomethacin, in HIGH and moderate intensity (MOD) trained and sedentary rats. Data are means±SD. Different curve-shapes of vasorelaxation upon accumulating doses of acetylcholine; HIGH and MOD vs. sedentary: *$p < 0.01$, No difference occurred between HIGH and MOD. Different relaxation at given doses of acetylcholine; HIGH vs. sedentary: †$p < 0.01$, ‡$p < 0.05$; MOD vs. sedentary: §$p < 0.01$, ‖$p < 0.05$.

Table 1
Echocardiography

	Sedentary	MOD	HIGH	Kruskal–Wallis
Diastolic LV diameter, mm	7.18 ± 0.34	7.45 ± 0.47	7.36 ± 0.37	0.22
Systolic LV diameter, mm	4.88 ± 0.45	4.81 ± 0.51	4.93 ± 0.38	0.82
Diastolic LV posterior wall thickness, mm	1.85 ± 0.16	1.96 ± 0.21	2.04 ± 0.13	0.06
Systolic LV posterior wall thickness, mm	2.63 ± 0.25	2.84 ± 0.20†	2.91 ± 0.17*	0.02
Diastolic intraventricular septum thickness, mm	1.96 ± 0.15	1.85 ± 0.16	2.05 ± 0.10‡	0.04
Systolic intraventricular septum thickness, mm	2.98 ± 0.27	2.89 ± 0.20	3.14 ± 0.24	0.11
Fractional shortening, %	32.2 ± 5.2	35.5 ± 4.6	33.0 ± 4.0	0.52
Heart rate, beats min^{-1}	284 ± 14	258 ± 36	250 ± 18§	0.05
E-wave peak velocity, cm s^{-1}	60.2 ± 11.1	69.4 ± 4.6	64.9 ± 7.0	0.62
A-wave peak velocity, cm s^{-1}	24.2 ± 4.3	27.8 ± 6.8	20.5 ± 6.1	0.26
E-wave deceleration time, cm s^{-1}	54.9 ± 11.1	50.9 ± 19.9	42.8 ± 8.0	0.21

Echocardiography measurements of high-(HIGH) and moderate-intensity, (MOD) trained and sedentary rats. LV: left ventricle; E-wave: early LV filling; and A: LV filling after atrial contraction. Data are mean±SD. HIGH vs. sedentary: * $p < 0.01$. MOD vs. sedentary: † $p < 0.05$. HIGH vs. MOD: ‡ $p < 0.05$. HIGH vs. sedentary: § $p < 0.05$. Kruskal–Wallis between group p values are also presented.

benefit, whereas endothelium-dependent mechanisms seem to plateau with more moderate efforts.

4.1. Intensity of training program

Several publications report that cardiovascular effects vary with intensity or amount of exercise. Variation from high to low aerobic capacity probably represents a continuum from health to disease [8,33]. However, this is the first time the magnitude of cellular effects was compared at two different exercise intensities. By weekly VO_{2max} assessments, running speed was adjusted in order to keep relative exercise intensity constant at either 65%–70% (MOD) or 85%–90% (HIGH) of maximum aerobic capacity throughout the study. For comparison with human activity levels, these intensities translate into approximately 11–13 (fairly light to somewhat hard, e.g. brisk walking/light jogging) on the Borg rating of perceived exertion [11] for MOD and 15–17 (hard to very hard, e.g. strenuous running) for HIGH. Since both experimental groups performed the same number of

intervals, HIGH individuals exceeded MOD not only by exercise intensity, but also by amount of work performed, distance run, and oxygen consumed. Although the results might to some extent result from higher exercise volume, this would probably have negligible practical consequences in search of an optimal training regimen. To match exercise volume in HIGH individuals, MOD would have to increase the number of 8-minute running intervals progressively from 25% during the first week to 100% when VO_{2max} plateaus. Thus, increasing exercise intensity is a highly efficient way to increase cellular effects of physical conditioning.

4.2. Cardiomyocyte function

Our results provide strong evidence that cardiomyocyte size and function is a central determinant of aerobic capacity. Larger improvement of VO_{2max} with high versus moderate training intensity correlated closely with different changes in cellular features translating into physiological hypertrophy with larger ventricle volume (cardiomyocyte

Table 2
Body mass and cardiac weights

	Sedentary	MOD	HIGH	Kruskal–Wallis
Body mass before, g	249.3 ± 18.1	244.9 ± 15.3	251.5 ± 11.9	0.59
Body mass after, g	294.4 ± 18.0	296.8 ± 19.1	288.6 ± 19.4	0.58
Heart weight, mg	1283.9 ± 251.6	1334.7 ± 191.3	1363.9 ± 224.6	0.68
Heart weight, mg g^{-1}	4.3 ± 0.7	4.5 ± 0.5	4.6 ± 0.7	0.52
Heart weight, mg g$^{-0.78}$	15.2 ± 2.4	15.7 ± 1.9	16.2 ± 2.4	0.56
LV weight, mg	996.7 ± 205.8	1011.8 ± 146.6	1050.7 ± 168.1	0.68
LV weight, mg g^{-1}	3.4 ± 0.5	3.4 ± 0.4	3.6 ± 0.5	0.60
LV weight, mg g$^{-0.78}$	11.8 ± 2.0	11.9 ± 1.5	12.5 ± 1.8	0.64
RV weight, mg	287.1 ± 54.1	318.3 ± 53.9	313.6 ± 74.4	0.31
RV weight, mg g^{-1}	1.0 ± 0.2	1.1 ± 0.1	1.1 ± 0.2	0.29
RV weight, mg g$^{-0.78}$	3.4 ± 0.5	3.7 ± 0.5	3.7 ± 0.8	0.30

Body mass before and after experimental period and post-mortem heart weights in trained and sedentary; heart weights after Langendorff perfusion (see Methods). Training lasted 10 weeks at either HIGH or MODerate intensity. Data are mean±SD. As the Kruskal–Wallis between group p-values show, no differences occurred between groups.
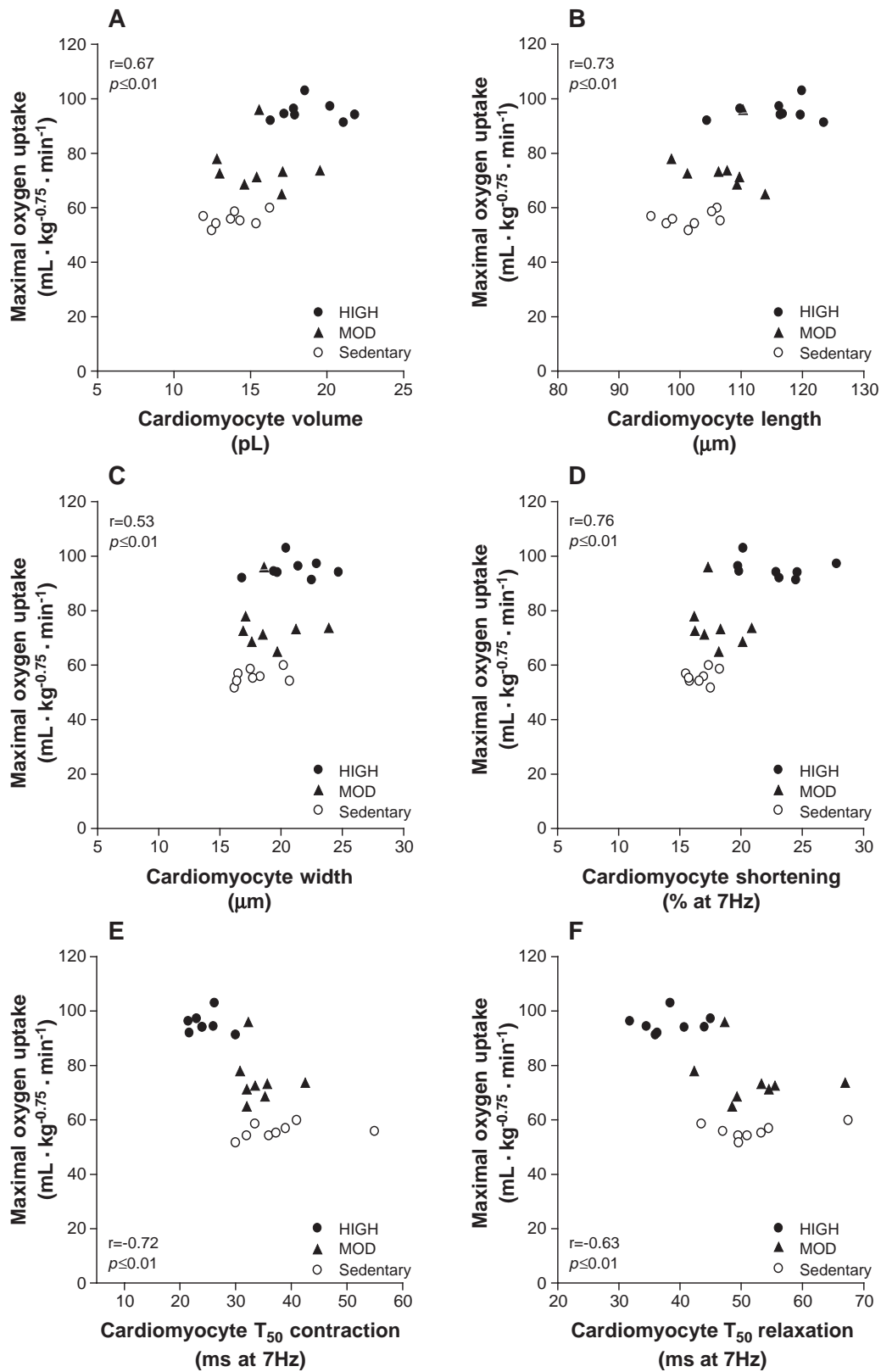
Fig. 6. Relationship between maximal oxygen uptake (VO_{2max}) and cardiomyocyte volume (panel A), length (panel B), width (panel C), fractional shortening (panel D), half-time systolic contraction (panel E), half-time diastolic relaxation (panel F), half-time to Ca^{2+} peak (panel G), half-time Ca^{2+} decay (panel H), Ca^{2+} sensitivity index (panel I), and acetylcholine-induced endothelial-dependent vasorelaxation (panel J) in HIGH and moderate intensity (MOD) trained and sedentary rats. Individual data with Pearson's correlation coefficients.
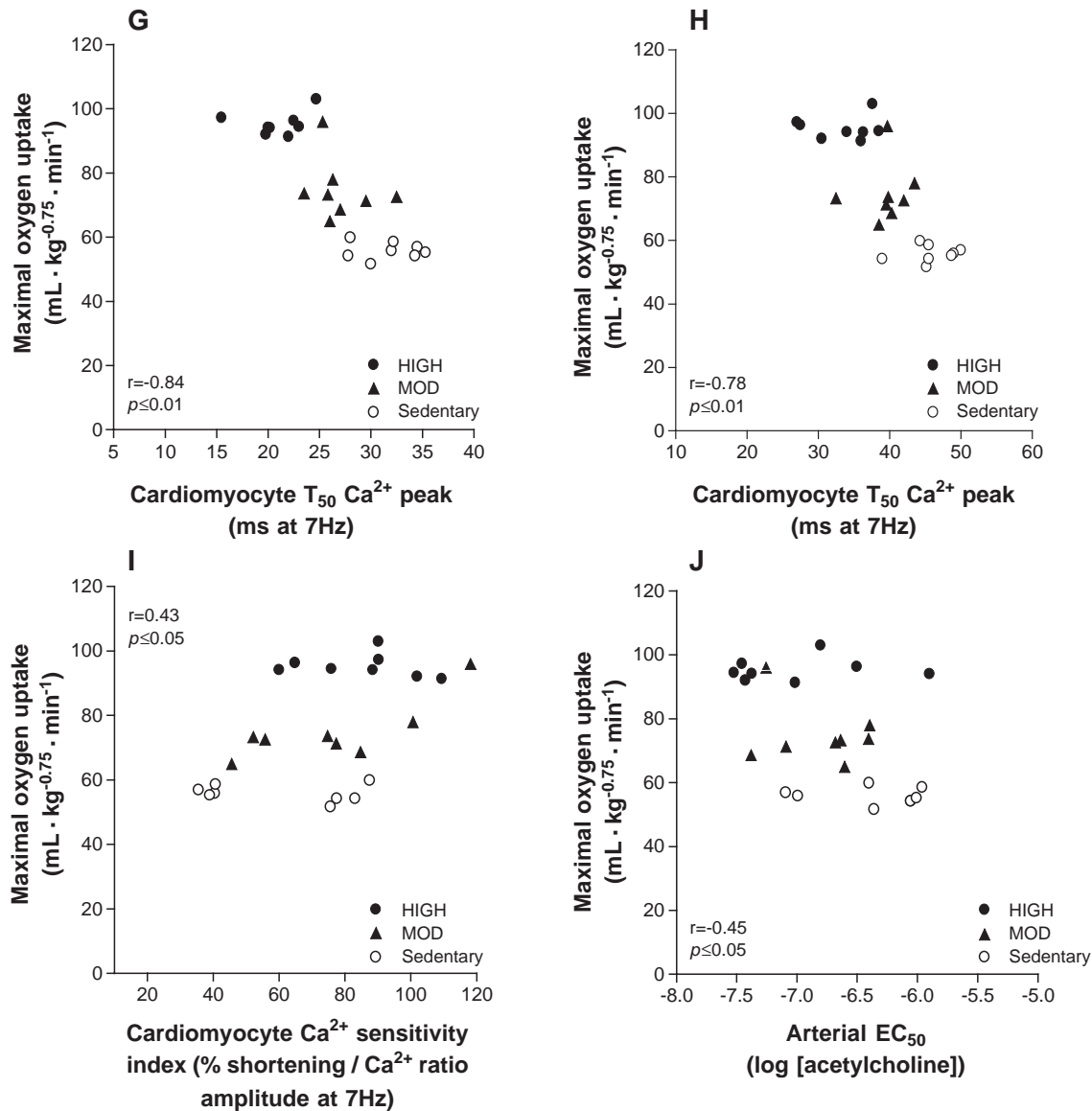
Fig. 6 (*continued*).

length and width), improved systolic contraction (cell shortening, time to 50% and peak Ca^{2+} and contraction), enhanced diastolic filling (time to 50% Ca^{2+} decay and relengthening) as well as increased Ca^{2+} sensitivity. These observations concur with recent experiments demonstrating similar correlations when VO_{2max} and cardiomyocyte characteristics changes over time, following the relatively slow onset with full effect 5–7 weeks after start of regular exercise training and the somewhat faster decay during 3–4 weeks of detraining [25]. Furthermore, they support the notion that increased stroke volume is a major component of adaptation to higher levels of aerobic exercise [34,35]. It is likely that increased cardiomyocyte size, contraction and relaxation all contribute mechanistically to higher stroke volume, cardiac output and VO_{2max}, even though only time to 50% of peak Ca^{2+} and time to 50% relengthening emerged out of the statistical analysis. Since all cardiomyo-

cyte variables were closely correlated, it is expected that only one or two come out as significant in multiple regression. It is interesting to note that cardiomyocyte function rather than merely size seems to be more strongly associated with aerobic capacity.

Cardiomyocyte contraction and relaxation are linked to the sarcoplasmatic reticulum Ca^{2+} ATPase (SERCA2) and its regulator phospholamban, both of which increase with regular exercise [19]. SERCA2 removes the main bulk of Ca^{2+} from the cytosol (Ca^{2+} decay), and restores sarcoplasmatic reticulum Ca^{2+} load before the next contraction cycle [36]. However, effect sizes on Ca^{2+} sequestering may to some degree be species-dependent, as SERCA2 removes ~90% of cytosolic Ca^{2+} in rat, whereas the equivalent in man is only ~70% [36]. Thus, the magnitude of exercise-induced effects may differ between rat and man.

Ca^{2+} transient amplitude did not explain increased fractional shortening. This suggests that myofilament responsiveness to Ca^{2+} instead is the mechanism, as indicated by the Ca^{2+} sensitivity index and in line with previous results [19,24]. Previously, Ca^{2+} sensitivity measured directly in skinned cells corresponded to that of intact cells [19].

Parallel adaptations to exercise occur in experimental heart failure after myocardial infarction, except that the beneficial effects on cardiac morphology is reverse remodelling with reduced pathologic cardiomyocyte hypertrophy and less left ventricle dilatation [20]. Based on this evidence, our working hypothesis for an ongoing clinical study is that high versus moderate intensity exercise may yield differential effects on functional and structural cardiomyocyte remodelling in heart failure patients.

4.3. Endothelial function

Endothelial function and arterial compliance constitute an important regulatory mechanism in exercise, as arterial conductance allows increased cardiac output to skeletal muscle [35]. However; the present study does not confirm a strong correlation between VO_{2max} and endothelial function, as endothelium-dependent dilation does not account for better aerobic capacity in HIGH than MOD. Although endothelial function increased with regular exercise and correlated with VO_{2max}, its adaptation pattern was distinctly different from that of cardiac myocytes. Endothelium-dependent relaxation reached nearly full effect with moderate exercise-intensity; barely a weak trend for increased sensitivity to acetylcholine occurred between HIGH and MOD, whereas both were higher than sedentary. Moreover, previous experiments demonstrated a different time-course than VO_{2max}, as endothelium-dependent gain in sensitivity to acetylcholine was completely abated after less than two weeks of detraining [25]. Whether the lack of interdependence between VO_{2max} and endothelial function is present in individuals with dysfunctional endothelium remains to be determined.

Both direct dilatory responses (nitroprusside) and reaction to acetylcholine after nitric oxide synthase-blockade (L-NAME) were similar in all groups, confirming that differential sensitivity to acetylcholine is endothelium-dependent. This is in line with exercise-induced up-regulation of the endothelial nitric oxide synthase pathway [14]. The carotid artery was chosen because of its clinical relevance in systemic circulation and predisposition for atherosclerosis. Its exercise-induced changes in endothelial function are similar to those in aorta (unpublished results from our lab), as expected since uphill treadmill running is a full-body exercise.

4.4. Exercise and gender

The present study was performed in adult female rats, which is the standard model for long-term studies in our laboratory because confounding by changing body mass is markedly smaller than in males. Since both rat carotid artery [37] and cardiomyocyte [38] contain estrogen receptors that promote endothelium-dependent vasodilatation via nitric oxide and prostaglandin pathways [37], and since estrogen blunts diastolic Ca^{2+} transient decay and myocyte relaxation [39–41], the magnitude of effects observed may be influenced by gender. It is possible that the adaptive window is smaller for endothelial response in females, but wider for myocyte contractile responses, because of different initial levels. However, intensity-dependent training-induced cardioprotection via increased levels of Heat Shock Protein 70 [42] are smaller in females than males [43]. Nonetheless, data so far suggest that VO_{2max} and myocyte adaptations are similar between genders [18], whereas the question remains more open for the endothelium.

5. Conclusion

The present study supports the notion that central aspects of myocardial adaptation to exercise depend on intensity of training program. Treadmill running with intervals at 85%–90% of current VO_{2max} yielded substantially larger effects on physiological hypertrophy, cardiomyocyte contractility, Ca^{2+} handling and aerobic fitness than moderate exercise at 65%–70% of VO_{2max}. In contrast, full effect on endothelial function was induced by regular exercise at moderate intensity, as endothelium-dependent carotid artery dilation was similar with high and moderate training levels. Although both myocardial and endothelium-dependent factors correlate significantly with VO_{2max}, parallel improvement in cardiomyocyte hypertrophy and contractile function from moderate to high intensity indicates that myocardial mechanisms may be more important for increased aerobic fitness. It seems likely that beneficial effects of regular exercise result from several mechanisms that may depend differentially on intensity; those associated with myocardial function seem to require high intensity training over several weeks to be fully active, whereas endothelium-dependent effects may plateau at lower intensity, depending on gender, age, function at baseline and other background variables. Thus, exercise intensity may emerge as an important variable in future clinical investigations.

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