Eur J Appl Physiol (2013) 113:1081–1090
DOI 10.1007/s00421-012-2524-4

ORIGINAL ARTICLE

Combined whole-body vibration, resistance exercise, and sustained vascular occlusion increases PGC-1α and VEGF mRNA abundances

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Received: 29 April 2012 / Accepted: 8 October 2012 / Published online: 20 October 2012

Abstract We previously reported that high load resistance exercise with superimposed whole-body vibration and sustained vascular occlusion (vibroX) markedly improves cycling endurance capacity, increases capillary-to-fibre ratio and skeletal muscle oxidative enzyme activity in untrained young women. These findings are intriguing, since increases in oxidative muscle phenotype and endurance capacity are typically induced by endurance but not heavy resistance exercise. Here, we tested the hypothesis that vibroX activates genes associated with mitochondrial biogenesis and angiogenesis. Eight healthy, recreationally resistance-trained young men performed either vibroX or resistance exercise (RES) in a randomised, cross-over design. Needle biopsies (*M. vastus lateralis*) were obtained at rest and 3 h post-exercise. Changes in relative gene expression levels were assessed by real-time quantitative PCR. After vibroX, vascular endothelial growth factor and peroxisome proliferator-activated receptor-γ coactivator 1α mRNA abundances increased to 2- and 4.4-fold, respectively, but did not significantly change above resting values after RES. Other genes involved in mitochondrial biogenesis were not affected by either exercise modality. While vibroX increased the expression of hexokinase II, xanthine dehydrogenase, and manganese superoxide dismutase mRNA, there were no changes in these transcripts after RES. This study demonstrates that high load resistance exercise with superimposed whole-body vibration and sustained vascular occlusion activates metabolic and angiogenic gene programs, which are usually activated after endurance but not resistance exercise. Thus, targeted modification of high load resistance exercise by vibration and vascular occlusion might represent a novel strategy to induce endurance-type muscle adaptations.

Keywords Blood flow restriction · Endurance exercise · Ischaemia · PGC-1α · VEGF · Whole-body vibration

Communicated by Martin Flueck.

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Introduction

Recently, we showed that high load [70 % one repetition maximum (1RM), American College of Sports Medicine position stand 2009] resistance exercise with superimposed whole-body vibration and sustained vascular occlusion (vibroX) leads to marked improvements in cycling endurance capacity as well as increases in capillary-to-fibre ratio, skeletal muscle oxidative enzyme activity, and myosin heavy chain type 1 (MYH-1) fibre proportion in previously untrained young women (Item et al. 2011). These new findings are intriguing, since increases in oxidative muscle

phenotype and endurance capacity are typically induced by endurance exercise but not high load resistance exercise (Coffey and Hawley 2007; Holloszy and Coyle 1984; Tesch 1988). In fact, most of the longitudinal studies examining the effect of resistance training on skeletal muscle oxidative potential report a decrease or no change in oxidative potential after training (Table 3 in Tang et al. 2006; Wilkinson et al. 2008). Although in the untrained state, an acute bout of heavy resistance exercise can increase rates of mitochondrial protein synthesis, the same relative training load does not stimulate mitochondrial protein synthesis anymore after a training period, i.e. in the resistance-trained state (Wilkinson et al. 2008).

One transcriptional coactivator that can modulate both oxidative metabolism and angiogenesis is peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) (Arany et al. 2008; Geng et al. 2010; Puigserver and Spiegelman 2003; St-Pierre et al. 2003; Wu et al. 1999). PGC-1α docks on and coactivates the nuclear respiratory factors 1 and 2α (NRF-1 and NRF-2α) (Wu et al. 1999). These transcription factors regulate the expression of nuclear genes encoding mitochondrial proteins (Scarpulla 2002) and induce the expression of mitochondrial transcription factor A (Tfam) (Wu et al. 1999). PGC-1α also activates the transcription factor oestrogen-related receptor α (ERRα), which elicits a robust induction of vascular endothelial growth factor (VEGF) in cultured muscle cells and mouse skeletal muscle in vivo following hypoxia and limb ischaemia, respectively (Arany et al. 2008). Arany et al. (2008) concluded that PGC-1α has a critical function in the angiogenic response to ischaemia and they speculated that the PGC-1α/ERRα pathway also mediates exercise-induced neovascularisation. Indeed, it has recently been shown that in rodent skeletal muscle, exercise-induced PGC-1α regulates VEGF (Chinsomboon et al. 2009; Geng et al. 2010). Notably, the induction of VEGF by PGC-1α has been shown to occur independent of hypoxia-inducible factor-1α (HIF-1α) (Arany et al. 2008), a well-known hypoxia-induced transcription factor regulating VEGF expression (Wenger 2002).

Since we showed that vibroX promotes capillarisation and increases in oxidative enzymes (Item et al. 2011), and since vibration and vascular occlusion—two distinguished/important features of vibroX—have been shown to activate VEGF expression and promote capillarisation in various settings (Gustafsson et al. 1999, 2002; Richardson et al. 1999; Sundberg 1994), we hypothesised that a single bout of vibroX should activate genes associated with mitochondrial biogenesis and angiogenesis. To test this hypothesis, we analysed *M. vastus lateralis* mRNA abundance of several genes involved in mitochondrial biogenesis and angiogenesis 3 h after an acute bout of vibroX, and compared them to resting levels. We also compared mRNA levels after vibroX with those obtained after high load resistance exercise alone, i.e. without vibration and sustained vascular occlusion, because we hypothesised that (1) mRNA responses after vibroX should be stronger than after resistance exercise alone, and (2) no significant increase in metabolic and angiogenic mRNA abundance should occur after high load resistance exercise in young, recreationally resistance-trained men. Furthermore, we measured gene transcripts involved in the oxidative stress response, since it has been shown that PGC-1α mRNA expression after a single bout of endurance exercise is influenced by the level of reactive oxygen species (ROS) (St-Pierre et al. 2006).

Methods

Participants

Eight men of age 23.1 years (SD 2.8) participated in this study. Participants weighed 75.3 kg (SD 9.2), their height was 1.81 m (SD 0.04), and their relative body fat content was 15.8 % (SD 2.4). All study participants were healthy, asymptomatic, non-smoking, and recreationally active. They were all familiar with resistance exercise. We chose to recruit resistance-trained participants to eliminate the potential of acute responses on mitochondrial biogenesis due to the novelty of a resistance exercise stimulus (Wilkinson et al. 2008). Parallel back squat 1 RM of study participants was 121 kg (SD 18). After completing a routine health questionnaire, the participants were informed about the applied procedures and the associated risks. Informed written consent was obtained from all participants. The experimental protocol was approved by the ethics committee of the canton of Zurich, and the study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki for human experimentation.

Trial design

Participants performed two trials, separated by two weeks. Each trial consisted of a bout of parallel back squat resistance exercise [RES; two sets, 70 % 1 RM (American College of Sports Medicine position stand 2009), 6–10 RM to failure, see Exercise protocols] or parallel back squats with superimposed whole-body vibration and sustained vascular occlusion [vibroX; two sets, 70 % 1 RM (American College of Sports Medicine position stand 2009), 6–10 RM to failure, see Exercise protocols]. Exercise sessions were administered in a randomised, cross-over fashion. Before the first exercise session, we performed baseline measurements (1 RM and body composition), a familiarisation session (exercise modalities and associated

procedures), and a baseline biopsy, all sessions separated by one week. Participants were asked to refrain from vigorous physical activity for 24 h prior to all sessions accomplished in this study. The evening (12 h) before the baseline biopsy and exercise sessions, participants were fed a standardised meal consisting of 3 g carbohydrate kg^{-1} body mass, 0.5 g protein kg^{-1} body mass, and 0.3 g fat kg^{-1} body mass.

Exercise protocols

VibroX has been previously described in detail (Item et al. 2011). In brief, it was comprised of loaded (Multipower®, Technogym, Gambettola, Italy) parallel back squats performed on a Galileo® side-alternating vibration plate (Novotec, Pforzheim, Germany) oscillating at 30 Hz, and superimposed vascular occlusion (Fig. 1). Load magnitude (70 % of the individual 1 RM) was adjusted from prior knowledge (familiarisation) in order to induce volitional failure within 60–100 s of exercise (6–10 RM to failure). Parallel back squats were performed over the individual's maximal range of motion, usually from near complete extension to a knee angle of slightly less than 90° in 4 s (eccentric action) and back to near complete extension in 4 s (concentric action). Transition from eccentric to concentric action was performed smoothly within 1–2 s. Volitional exhaustion (task failure) was defined as the point in time where the participants failed to extend their legs. Vascular occlusion was induced by inflating tourniquet cuffs (width 0.09 m, length 0.76 m; VBM, Sulz a.N., Germany) affixed to the inguinal fold region of the thigh to approximately 200 mmHg (26.7 kPa). The suprasystolic pressure employed here was the highest pressure that was tolerated by the participants in this setting. The exercise regimen consisted of two sets, each set with a duty cycle of 4 min and 1 min off. During one duty cycle, participants performed loaded squats with superimposed whole-body vibration and vascular occlusion until volitional failure. Subsequently, tourniquet cuffs remained inflated until the 4 min of the duty cycle were complete. The cuffs were then deflated to a pressure of 100 mmHg (13.3 kPa), and participants rested for 1 min before cuffs were reinflated for the second set. The load magnitude was reduced by approximately 10 % points for the second set in order to keep exercise time (i.e. time under tension, Toigo and Boutellier 2006) constant.

During RES, solely parallel back squats were performed on the Multipower® device (Technogym, Gambettola, Italy). That is, during RES squats were not combined either with vibration or occlusion. After volitional exhaustion (6–10 RM to failure), participants rested for 4 min standing still until the second set started. Thus, for RES, we adopted exactly the same time interval between the two squat sets as for vibroX, because we wanted to exclude any possible effects of time on mRNA responses.

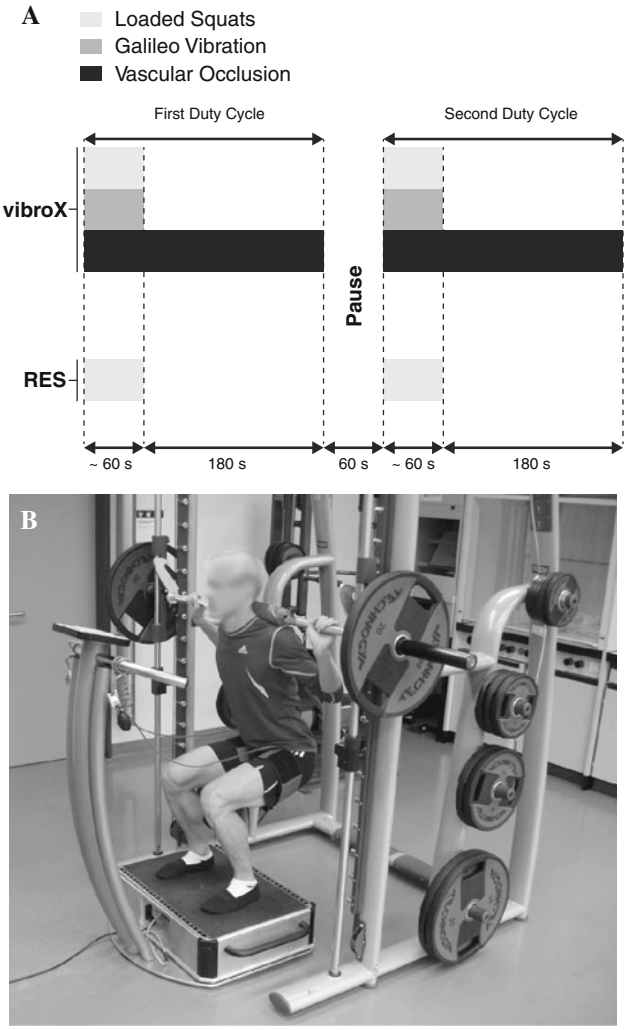

Fig. 1 Schematic representation of the exercise setup (**a**) and an illustration of the exercise regimen of vibroX comprising parallel back squats performed on a side-alternating vibration plate with superimposed vascular occlusion (**b**). *RES* resistance exercise, *vibroX* Galileo® vibration + resistance exercise + vascular occlusion

Skeletal muscle biopsies

We obtained percutaneous biopsies from the nondominant *M. vastus lateralis* at baseline and 3 h after exercise using a 6 mm Bergström needle (Dixons Surgical Instruments, Essex, UK) with suction applied. This time point was chosen due to comparability with other studies. All biopsies were harvested via separate incisions (separated by 3–4 cm) ~2 cm laterally from the mid-third portion (~15 cm in length) of the longitudinal skin surface line (connecting the palpable great trochanter and the patella in supine position with thigh and knee extended, measuring ~45 cm), corresponding to the underlying mid-third

M. vastus lateralis. Biopsy samples were immediately frozen in liquid nitrogen and stored at −80 °C until mRNA was analysed.

RNA extraction and mRNA analyses

Total RNA from *M. vastus lateralis* samples was extracted by using the RNeasy fibrous tissue minikit (Qiagen, Basel, Switzerland). The purity of the resulting RNA was assessed by the 260/280 nm ratio. First-strand complementary DNA synthesis was performed with 200 μg total RNA by using reverse transcriptase, and mRNA abundance was determined by real-time quantitative PCR by using a Sybr-Green® qPCR reagent kit (Sigma, Buchs, Switzerland) in combination with the MX3000P light cycler (Stratagene, Amsterdam, The Netherlands). Initial template concentrations of each sample were calculated by comparison with serial dilutions of a calibrated standard. Equal input levels were verified by determining ribosomal protein L28 mRNA abundance, and data were expressed as ratios relative to L28 mRNA expression. Sequences of the primers are given in Table 1.

Dual-energy X-ray absorptiometry (DXA)

We used dual-energy X-ray absorptiometry (Lunar iDXA™, GE Healthcare, Madison, WI, USA) to determine whole-body lean and fat masses according to the outlines of the manufacturer.

Determination of squat 1 RM

One RM measurements of parallel back squats were performed as during vibroX (described in "exercise protocols"). A short warm-up was followed by three repetitions at 70 % anticipated maximum. Three to five subsequent attempts were made to determine 1 RM, with a 5-min rest interval between squats.

Statistical analyses

Data are presented as mean and SD. To obtain normally distributed data, mRNA copy numbers were log-transformed. Relative mRNA levels were subjected to a two-way repeated measures ANOVA having 14 levels of gene (Table 1) and three levels of condition (rest, vibroX, and RES). For the three effects (two main effects: gene and condition; one interaction: gene x condition), the assumption of sphericity was tested using Mauchly's test. In the case of violation of the assumption of sphericity, degrees of freedom were corrected using Greenhouse–Geisser corrected estimates of sphericity. Contrasts were used to break down the main effects and the interaction term. Statistical significance was set at $P < 0.05$. Effect sizes for these contrasts were expressed as Pearson's correlation coefficient r, which in turn was calculated by using F-ratios and the residual degrees of freedom (Rosnow et al. 2000). Magnitude thresholds of 0.1, 0.3, and 0.5 for small, moderate, and large correlation coefficients were used according to Cohen (1988). The level of significance was set to $P < 0.05$. For all statistical analyses, SPSS 16.0 statistical software (SPSS, Chicago, IL, USA) was used.

Results

Skeletal muscle mRNA analyses

There was a significant ($P < 0.001$) main effect of the type of gene on mRNA expression level, F(2.97,

Table 1 PCR primer sequences for the measured gene transcripts

Gene	Accession no	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)
CS	BT007414	aggaagactgatccgcgata	catggacttgggcctttcta
ERRα	NM004451	tgccaattcagactctgtgc	cctcgagcatctccaagaac
HIF-1α	HSU22431	tcatccaagaagccctaacg	ttgtcttttgctccattcca
HKII	NM000189	tggctttgaagaccctcact	caaattctgtgcggaagtca
L28	NM000991	gcaattccttccgctacaac	tgttcttgcggatcatgtgt
LDHA	NM001135239	tgtgcctgtatggagtggaa	cccaaaatgcaaggaacact
MnSOD	M36693	ccctggaacctcacatcaac	agtcacgtttgatggcttcc
NRF-1	NM001040110	atgcagcagggagctacagt	atgctcacagggatctggac
PFKm	J05533	gtccctggctcagacttcag	ccttaacaccaagccccttt
PGC-1α	AF159714	caagccaaaccaacaactttatctct	cacacttaaggtgcgttcaatagtc
PHD3	NM022073	atcgacaggctggtcctcta	cttggcatcccaattcttgt
Tfam	NM003201	ggaaaaccaaaaagacctcgttcagctt	ttttcctgcggtgaatcaccctt
VEGF	NM001025366	ctacctccaccatgccaagt	tggtgatgttggactcctca
XD	NM000379	acacccaatctgggctacag	ccgggatcttgtaggtgcta

28.45) = 161.45. Further, there was a significant ($P < 0.001$) main effect of the type of condition on mRNA abundance, $F(1.71, 0.81) = 14.87$. Contrasts revealed that relative mRNA levels for vibroX were higher than rest, $F(1, 7) = 99.95$, $r = 0.97$, and RES, $F(1, 7) = 17.02$, $r = 0.84$. Finally, there was a significant ($P = 0.04$) interaction effect between the type of gene and the type of condition, $F(2.26, 5.21) = 3.03$. This indicates that the condition had different effects on relative mRNA expression levels depending on the type of gene. Contrasts comparing all gene types to their baseline (L28), the conditions vibroX and RES to their baseline (rest), and vibroX to RES revealed the following significant interactions:

Mitochondrial biogenesis

There was a significant and strong effect of vibroX on PGC-1α gene expression, $F(3.27, 7) = 345.04$, $r = 0.99$. PGC-1α mRNA abundance after vibroX was significantly higher as compared to RES (Fig. 2a), and this contrast did yield a large effect size, $F(1.15, 7) = 6.63$, $r = 0.56$. PGC-1α mRNA abundance increased 4.4-fold above rest after vibroX with no significant increase after RES (Fig. 2a). We observed no significant increase in the mRNA abundance of transcription factors associated with mitochondrial biogenesis, such as ERRα, NRF-1, and Tfam for either training modality (Fig. 2b–d).

Angiogenesis and oxygen sensing

Contrasts revealed large effect sizes for both vibroX versus RES, $F(0.54, 7) = 3.27$, $r = 0.56$ and vibroX versus rest $F(0.54, 7) = 3.27$, $r = 0.56$. VibroX caused a significant twofold increase in VEGF mRNA abundance, while no increase above resting levels was observed after RES (Fig. 3a). No changes in HIF-1α and HIFα prolyl hydroxylase domain 3 (PHD3) mRNA expression were detected subsequent to vibroX and RES (Fig. 3b, c).

Metabolic key enzymes

The HKII expression level after vibroX was significantly higher than after RES (Fig. 4a). Contrasts yielded large effect sizes, $F(0.54, 7) = 3.27$, $r = 0.56$ and $F(0.54, 7) = 3.27$, $r = 0.56$ for vibroX versus RES and vibroX versus rest, respectively. Hexokinase II (HKII) mRNA abundance was significantly increased by 2.5-fold 3 h after vibroX but remained unchanged after RES (Fig. 4a). The mRNA levels of other metabolic key enzymes such as citrate synthase (CS), lactate dehydrogenase A (LDHA), and phosphofructokinase (PFKm) were not affected by either exercise modality (Fig. 4b–d).

Oxidative stress

The mRNA abundance of xanthine dehydrogenase (XD) increased significantly after vibroX (2.3-fold) but not after RES (Fig. 5a). This effect size was large, $F(0.69, 7) = 15.51$, $r = 0.83$. However, there was no significant difference in XD mRNA abundance between vibroX and RES (Fig. 5a). Furthermore, manganese superoxide dismutase (MnSOD) mRNA abundance significantly increased after vibroX (1.6-fold) but remained unchanged after RES (0.8-fold) (Fig. 5b). MnSOD expression changes were significantly different between groups ($P = 0.001$) (Fig. 5b).

Discussion

The principal findings of the present study were that (1) 3 h post-exercise, VEGF, PGC-1α, HKII, XD, and MnSOD mRNA abundances were increased for vibroX but not RES, and (2) for all of these mRNAs, contrasts comparing vibroX versus rest and vibroX versus RES did yield large effect sizes. These results indicate that the addition of side-alternating whole-body vibration and sustained vascular occlusion altered the molecular response to loaded squats by inducing genes that are usually activated only after endurance-type exercise and/or oxidative stress. Furthermore, the herein reported findings coincide with the observed long-term adaptations following repeated vibroX exercise. Indeed, we have previously shown that 5 weeks of vibroX training increases capillary-to-fibre ratio, skeletal muscle oxidative enzyme activity, MYH-1 fibre proportion, and endurance capacity (Item et al. 2011). Altogether, these findings indicate that repeated vibroX training mediates a shift towards a more oxidative and fatigue resistant muscle. The results of this study now point to a major role of PGC-1α and VEGF in mediating these cellular adaptations.

VEGF plays an essential role in exercise-induced angiogenesis in skeletal muscle (Olfert et al. 2010), and reduced oxygen partial pressure represents a major stimulus for inducing VEGF (Forsythe et al. 1996). However, it remains unclear as to whether endurance exercise in systemic hypoxia or with restricted blood flow, which is assumed to induce a hypoxic condition within the involved muscles, potentiates the VEGF response (Gustafsson et al. 1999, 2002; Richardson et al. 1999). Questioning the importance of a reduced cellular partial oxygen pressure as a stimulus for an exercise-induced increase in VEGF expression, Richardson et al. (1999) suggested that there exists an intracellular oxygen partial pressure threshold, beyond which no greater angiogenic stimulus is produced, and that endurance exercise in normoxia can achieve this threshold. Importantly, the presumed hypoxic tissue

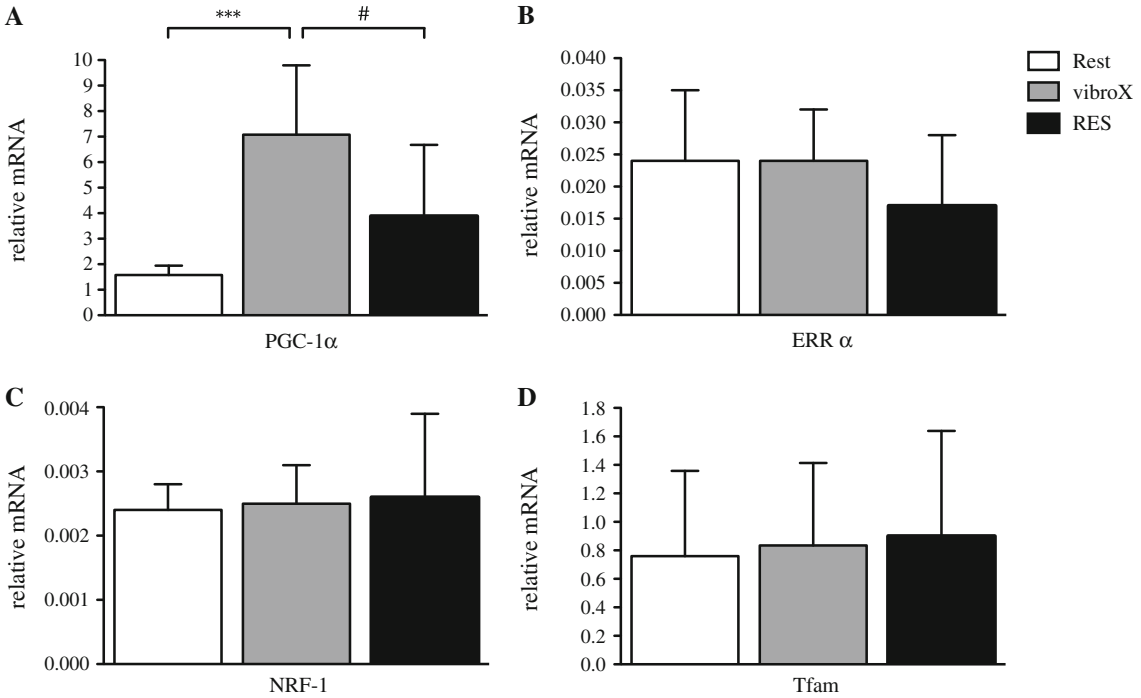

Fig. 2 Alterations in PGC-1α (**a**) ERRα (**b**) NRF-1 (**c**) and Tfam (**d**) mRNA abundances 3 h after a single bout of vibroX or RES relative to resting levels in eight participants. *Bars* and *error bars* represent mean values and SD, respectively. *ERRα* oestrogen-related receptor *α*, *NRF-1* nuclear respiratory factor 1, *PGC-1α* peroxisome proliferator-activated receptor-*γ* coactivator 1α, *RES* resistance exercise, *Tfam* mitochondrial transcription factor A, *vibroX* Galileo® vibration + resistance exercise + vascular occlusion. #Significantly different between vibroX and RES, $P < 0.05$; ***significantly different from rest, $P < 0.001$

condition induced in vibroX was achieved with vascular occlusion, which is—in contrast to normobaric hypoxia—associated with both limited arterial inflow and impeded venous outflow, thus resulting in deprivation of both oxygen and nutrients as well as a decreased transport of waste products away from the muscles. This increased metabolic cell stress (even more increased with sustained vascular occlusion) likely influenced the observed transcriptional responses.

In cell culture, it has been shown that hypoxia increases VEGF mRNA abundance through transcription factor HIF-1-activated VEGF promoter activity (Forsythe et al. 1996). HIF-1 is a heterodimeric transcription factor composed of HIF-1α and HIF-1*β* subunits and its activity is post-translationally regulated by HIF-1α stabilisation in hypoxic conditions. Similar to hypoxia, endurance exercise has been shown to induce VEGF mRNA and HIF-1α protein abundances (Ameln et al. 2005; Gustafsson et al. 1999). Notably, HIF-1α mRNA expression in human skeletal muscles remains unaffected by endurance exercise (Ameln et al. 2005; Gustafsson et al. 1999). VibroX neither increased HIF-1α mRNA abundance nor the expression of HIF-1α target genes (e.g. PHD3, LDHA, PFKa) (Figs. 3b, c, 4c, d), which led us to speculate that VEGF expression was induced in a HIF-1 independent manner.

Instead of HIF-1α, two other possible mechanisms are more likely to explain why VEGF was induced after vibroX but not RES: (1) increased PGC-1α and/or (2) shear stress. It has been reported that PGC-1α regulates VEGF in rodent skeletal muscles (Chinsomboon et al. 2009; Geng et al. 2010), most likely in a HIF-1α independent way (Arany et al. 2008). Recently, it has also been shown that the acute response of PGC-1α mRNA to high-intensity exercise depends on exercise intensity (Egan et al. 2010; Nordsborg et al. 2010) and/or muscle fibre activation pattern (Godin et al. 2010), indicating that high power output and increased metabolic cell stress are key factors for inducing PGC-1α expression. Since PGC-1α expression was increased after vibroX only, superimposed sustained vascular occlusion and whole-body vibration might have triggered this response. In fact (sustained) vascular occlusion further increases metabolic cell stress (e.g. accumulation of metabolites and ions) (Suga et al. 2010) and vibration leads to specific increases in ATP turnover (Wang and Kerrick 2002) and energy demand (Rittweger et al. 2001, 2002). Since vibroX and RES were both based on high load resistance exercise (70 % 1 RM), leading to complete motor unit recruitment of the leg muscles when performed to exhaustion (De Luca et al. 1996), it seems unlikely that a different muscle fibre activation pattern

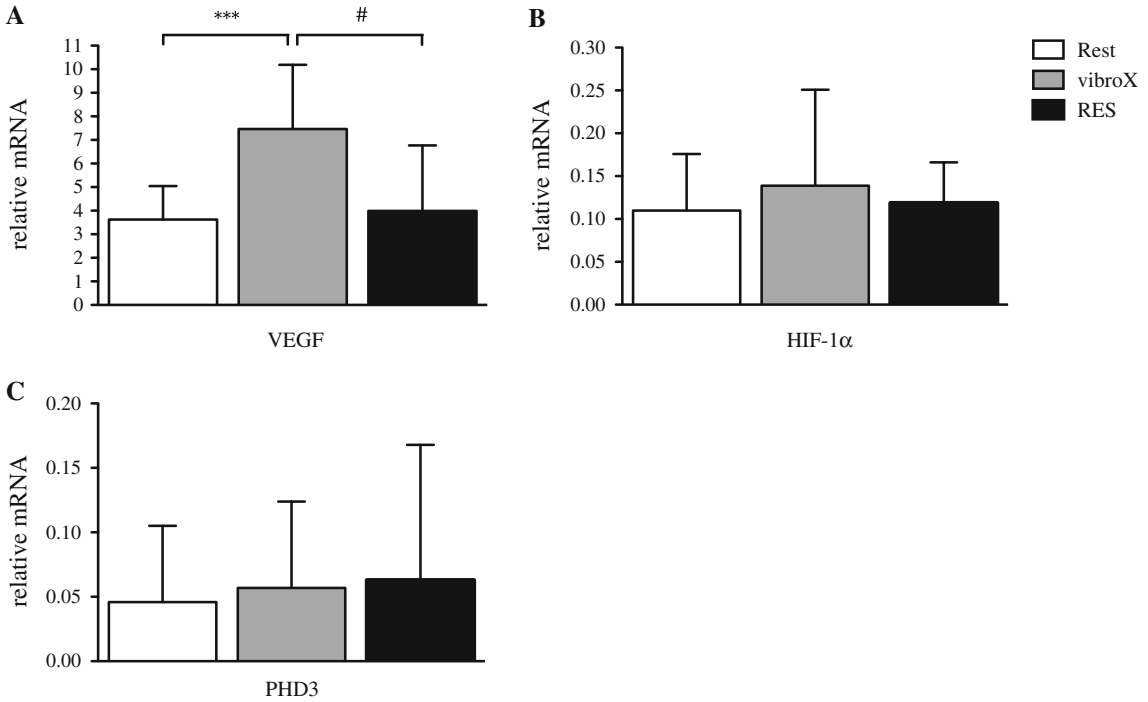

Fig. 3 Alterations in VEGF (**a**), HIF-1α (**b**), and PHD3 (**c**) mRNA abundances 3 h after a single bout of vibroX or RES relative to resting levels. *Bars* and *error bars* represent mean values and SD, respectively. *HIF-1α* hypoxia-inducible transcription factor 1α, *PHD3* prolyl hydroxylase domain 3, *RES* resistance exercise, *VEGF* vascular endothelial growth factor, *vibroX* Galileo® vibration + resistance exercise + vascular occlusion. #Significantly different between vibroX and RES, $P < 0.05$; ***significantly different from rest, $P < 0.001$

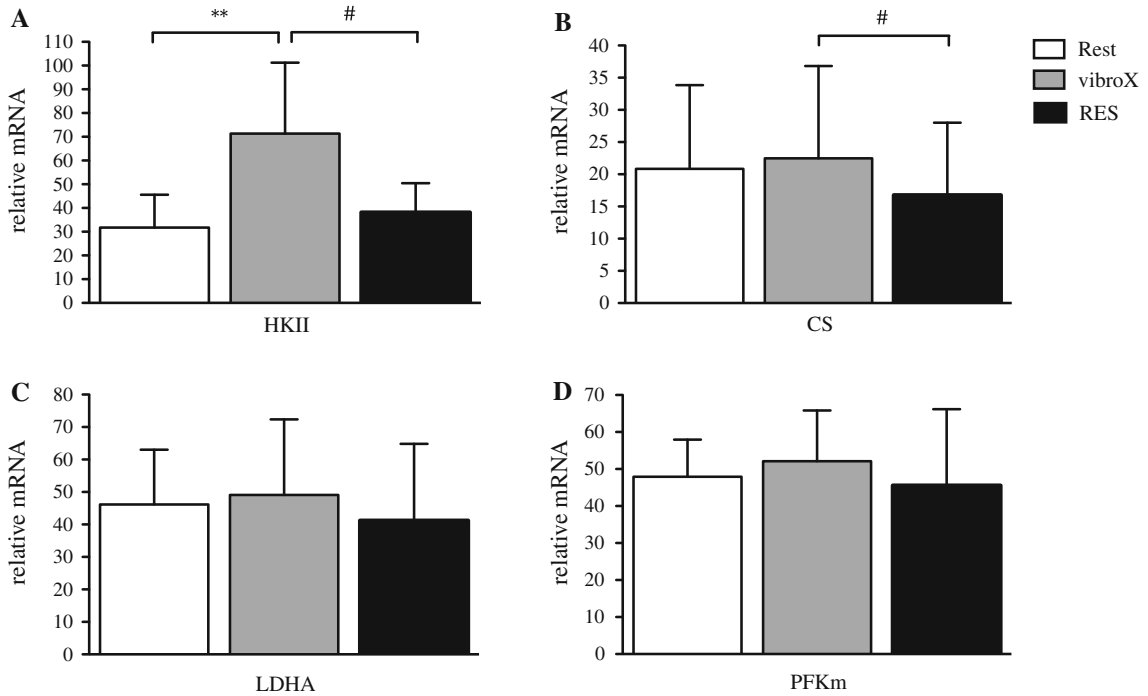

Fig. 4 Alterations in HKII (**a**), CS (**b**), LDHA (**c**), and PFKm (**d**) mRNA abundances 3 h after a single bout of vibroX or RES relative to resting levels. *Bars* and *error bars* represent mean values and SD, respectively. *CS* citrate synthase, *HKII* hexokinase II, *LDHA* lactate dehydrogenase A, *PFKm* phosphofructokinase muscle type, *RES* resistance exercise, *vibroX* Galileo® vibration + resistance exercise + vascular occlusion. #Significantly different between vibroX and RES, $P < 0.05$; **significantly different from rest, $P < 0.01$

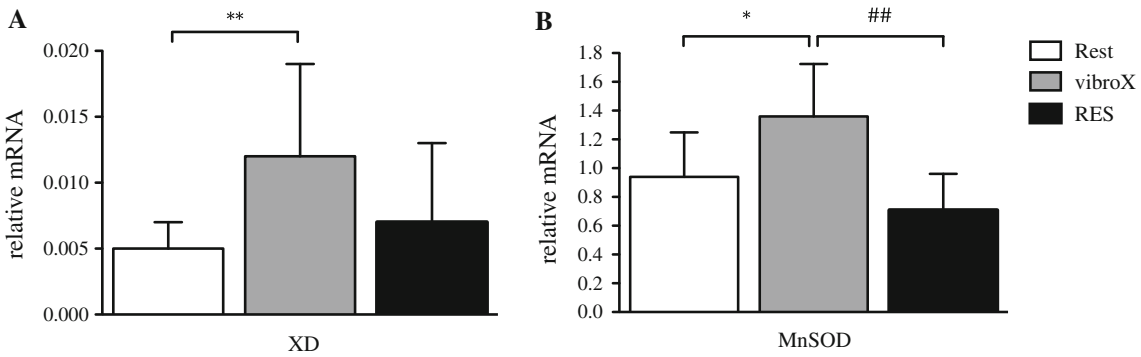

Fig. 5 Alterations in XD (**a**) and MnSOD (**b**) mRNA abundances 3 h after a single bout of vibroX or RES relative to resting levels. *Bars* and *error bars* represent mean values and SD, respectively. *MnSOD* manganese superoxide dismutase, *RES* resistance exercise, *vibroX* Galileo® vibration + resistance exercise + vascular occlusion, *XD* xanthine dehydrogenase. ##Significantly different between vibroX and RES, $P < 0.01$; *,**significantly different from rest; $P < 0.05$, $P < 0.01$

accounted for the different PGC-1α expression after vibroX and RES.

Concomitant to the increase in PGC-1α expression, we found that MnSOD and XD mRNA abundances were both elevated only after vibroX (Fig. 5), which indicated the presence of oxidative stress. Since it has been shown that PGC-1α signalling is sensitive to ROS (Kang et al. 2009; St-Pierre et al. 2006), we suspect that the elevated oxidative stress after vibroX led to the observed increase in PGC-1α expression. The increase in XD mRNA after vibroX but not RES further highlighted the specific roles of (sustained) vascular occlusion and vibration in the generation of oxidative stress. XD is the dehydrogenase form of xanthine oxidase (XO), which is localised in the vascular walls of skeletal muscles but not in myofibres itself (Hellsten-Westing 1993). This fact is highlighted by the low XD mRNA copy numbers (Fig. 5a). XO is capable of producing superoxide radicals (Vina et al. 2000) and has been identified as a source of superoxide in reperfusion injury. Thus, it can be speculated that similar to reperfusion injury, vibroX led to the conversion of XD to XO in endothelial cells of the vascular walls, and subsequently to the production of ROS, which in turn triggered the expression of PGC-1α in skeletal muscle cells. Alternatively or concomitantly, VEGF expression after vibroX might also have been driven by mechanical and shear stresses (Prior et al. 2004). In this regard, vibration exercise has been reported to induce shear stress at the wall of vessels leading to increases in circulating blood VEGF protein (Suhr et al. 2007). Thus, based on our findings it appears that both sustained vascular occlusion and/or the associated reperfusion, and whole-body vibration contributed to the induction of VEGF mRNA abundance after vibroX.

Although PGC-1α expression was significantly increased after vibroX, ERRα, NRF-1, and Tfam remained unaffected (Fig. 2b–d). These findings are in line with reports of other studies in humans, where it has also been observed that despite the marked increase in PGC-1α, NRF-1 and Tfam remain unaffected (Norrbom et al. 2004; Pilegaard et al. 2003). In contrast, in C2C12 myotubes overexpressing PGC-1α, NRF-1 and NRF-2α mRNA are dramatically increased (Wu et al. 1999). Moreover, HKII, a gene that is often associated with adaptations to endurance training, was only up-regulated after vibroX (Fig. 4a), while other metabolic key enzymes such as CS, LDHA, and PFKm (Fig. 4b–d) were not increased after vibroX or RES. Pilegaard et al. (2003) also found that HKII is the only metabolic key enzyme whose expression is increased after 3 h of knee extension exercise. These authors suggest that genes display a different responsiveness to exercise and that there exists a temporal hierarchy to the transcriptional activation of some genes after a single exercise stimulus. In order to gain more insight into the temporal changes in mRNA abundances following vibroX, time course analysis are warranted. Nevertheless, the fact that the observed responses (direction and magnitude) for the previously described key metabolic genes are in line with those observed by other investigators supports this concept.

The present study aimed to investigate whether specific gene transcripts are differentially regulated after a single bout of vibroX compared to traditional high load resistance exercise and thus, whether the molecular responses coincide with the structural and functional adaptations after a period of vibroX training, as reported in an earlier study (Item et al. 2011). In order to be able to draw more mechanistic conclusions, the contribution of each of the four superimposed stimuli, i.e. high load resistance exercise, side-alternating vibration, vascular occlusion during contraction, and sustained vascular occlusion will need to be deciphered. The present data does not provide any insights into the upstream molecular pathways and furthermore, we cannot exclude that other genes than PGC-1α additionally play a role in mediating the presented gene

expression regulation and the long-term adaptations as described in Item et al. (2011). Other signalling pathways (e.g. calcineurin-NFAT, AMPK) may also be involved in promoting the cellular adaptations evoked by vibroX.

Conclusion

We have reported that combined whole-body vibration, resistance exercise and sustained vascular occlusion strongly activated gene programs typically linked to endurance exercise in recreationally resistance-trained men. VibroX increased the expression of VEGF mRNA possibly through ROS-activated PGC-1α and probably in a HIF-1α independent manner. We speculated that vascular occlusion and increased shear stress likely induced XO in endothelial cells of the vascular walls, which subsequently induced the production of ROS. The presented molecular responses further strengthen the notions that vibration and (sustained) vascular occlusion can lead to shear stress and metabolic cell stress, respectively. Consequently, the addition and/or combination of these stimuli to/with classical exercise modalities such as high load resistance exercise or endurance exercise might represent a new avenue to "tailor" the molecular responses to training.

Acknowledgments We thank the participants for their effort and time commitment. This work was supported by the University of Zurich research priority program "Integrative Human Physiology" and the Zürcher Kantonalbank (ZKB). The Galileo® vibration plate was kindly provided by Novotec, Pforzheim, Germany.

Conflict of interest The authors declare no conflict of interests.

Ethical standard The experimental protocol was approved by the ethics committee of the canton of Zurich, and the study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki for human experimentation.

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