

Six weeks of a polarized training-intensity distribution leads to greater physiological and performance adaptations than a threshold model in trained cyclists

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Neal CM, Hunter AM, Brennan L, O'Sullivan A, Hamilton DL, De Vito G, Galloway SDR. Six weeks of a polarized training-intensity distribution leads to greater physiological and performance adaptations than a threshold model in trained cyclists. *J Appl Physiol* 114: 461–471, 2013. First published December 20, 2012; doi:10.1152/jappphysiol.00652.2012.—This study was undertaken to investigate physiological adaptation with two endurance-training periods differing in intensity distribution. In a randomized crossover fashion, separated by 4 wk of detraining, 12 male cyclists completed two 6-wk training periods: 1) a polarized model [6.4 (± 1.4 SD) h/wk; 80%, 0%, and 20% of training time in low-, moderate-, and high-intensity zones, respectively]; and 2) a threshold model [7.5 (± 2.0 SD) h/wk; 57%, 43%, and 0% training-intensity distribution]. Before and after each training period, following 2 days of diet and exercise control, fasted skeletal muscle biopsies were obtained for mitochondrial enzyme activity and monocarboxylate transporter (MCT) 1 and 4 expression, and morning first-void urine samples were collected for NMR spectroscopy-based metabolomics analysis. Endurance performance (40-km time trial), incremental exercise, peak power output (PPO), and high-intensity exercise capacity (95% maximal work rate to exhaustion) were also assessed. Endurance performance, PPOs, lactate threshold (LT), MCT4, and high-intensity exercise capacity all increased over both training periods. Improvements were greater following polarized rather than threshold for PPO [mean ($\pm$ SE) change of 8 (± 2)% vs. 3 (± 1)%, $P < 0.05$], LT [9 (± 3)% vs. 2 (± 4)%, $P < 0.05$], and high-intensity exercise capacity [85 (± 14)% vs. 37 (± 14)%, $P < 0.05$]. No changes in mitochondrial enzyme activities or MCT1 were observed following training. A significant multilevel, partial least squares-discriminant analysis model was obtained for the threshold model but not the polarized model in the metabolomics analysis. A polarized training distribution results in greater systemic adaptation over 6 wk in already well-trained cyclists. Markers of muscle metabolic adaptation are largely unchanged, but metabolomics markers suggest different cellular metabolic stress that requires further investigation.

exercise; metabolism; metabolomics; skeletal muscle

UNDERSTANDING THE OPTIMAL exercise training-intensity distribution to maximize adaptation and performance is important for athletes who try to gain a competitive advantage. In addition, a greater understanding of the interactions among exercise-intensity distribution, physiological stress, and adaptation could be important for achieving the optimal health benefits from physical activity in the general population. Exercise-intensity distribution is determined from the percentage of time spent exercising at low [*zone 1*, typically <65% of peak power

output (PPO), less than the lactate threshold (LT), <2 mM]; moderate [*zone 2*, ~65–80% of PPO, between LT and lactate turn point (LTP)]; and high [*zone 3*, typically >80% of PPO, >LTP, >4 mM] intensities (8, 29, 46). It has been suggested that two distinct exercise training-intensity distribution models are adopted by endurance athletes (46). First, a polarized training model (POL) that consists of a high percentage of exercise time at low exercise intensity (~75–80%) accompanied by little time at moderate intensity (~5–10%) with the remainder spent at high intensity (~15–20%). In contrast, the second model is a threshold training distribution (THR), in which moderate exercise intensity is the focus (typically 40–50% of training time) with relatively little or no high-intensity work and the balance of training time spent at low intensity.

It has been suggested by Seiler (47) and Laursen (32) that adopting a polarized intensity distribution may optimize adaptation to exercise while providing an acceptable level of training stress. Several studies have investigated adaptation to training at different intensities, with positive effects on LT and performance observed when a high proportion of training is conducted at low intensities (12, 13, 26). These studies suggest that the proportion of time in *zone 1* is a key aspect that drives endurance adaptations and performance outcomes. However, other studies (33, 57, 58) have observed increased PPO and mean power sustainable during a 40-km time trial (40-km TT) when high-intensity interval work (*zone 3* training) is incorporated into the schedules of already well-trained cyclists; i.e., when the cyclists adopted a more polarized training-intensity distribution. In addition, the change of intensity distribution toward a more polarized model has been shown to improve maximal oxygen consumption, running economy, and running performance in a case study of an international 1,500-m runner (27). Indeed, the powerful stimulus afforded by short-term, high-intensity interval work for promoting metabolic and performance adaptations has also been demonstrated in studies on trained-cyclist (51), healthy-active (52), and sedentary (23) men and women. These studies have shown significant increases in skeletal muscle oxidative capacity and mitochondrial function following only a few high-intensity interval exercise sessions, as well as improvements in markers of endurance performance. Thus the combination of a high proportion of time in *zone 1* along with *zone 3* interval work is likely to be a strong combination for optimal adaptations to training in endurance athletes, but to date, no study has directly compared the adaptations induced by POL vs. THR in already well-trained athletes.

An important aspect in adaptation to exercise is recovery and the ability to cope with the training stress. Seiler et al. (48)

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identified that recovery time from high-intensity training was not greater than from moderate-intensity training but that recovery time from low-intensity training was the shortest. Their data imply that recovery from a polarized training-intensity distribution would be better than recovery from a threshold intensity distribution. With new technologies, such as metabolomics that enable a more global overview of whole-body metabolic perturbations, the response to exercise-training stress, adaptation, and recovery can be studied in a more global manner.

Metabolomics technology has, in recent years, provided new insights in several fields of research, including toxicology, pharmacology, and human nutrition, and can aid identification of novel biomarkers (40). However, the application of metabolomics to exercise training has been under used in human exercise studies to date. There are only two cross-sectional human studies that have been published (11, 61), and both of these concluded that metabolomics is a promising tool for investigation of human responses to exercise. Therefore, the purpose of the present study was to compare the physiological adaptations and longitudinal metabolomics profile responses of well-trained male cyclists with training interventions that followed both a polarized and a threshold training-intensity distribution. We hypothesized that the polarized training-intensity distribution would lead to greater adaptive responses through a greater stimulus provided by the high-intensity interval exercise and the high proportion of training spent at low intensity. We also hypothesized that the metabolomics profile would provide new insights into understanding the training stresses induced by POL vs. THR in already well-trained humans.

METHODS

Twelve well-trained male cyclists were recruited from two local cycling clubs. The mean ($\pm$ SD) characteristics of the participants

were: age 37 ($\pm$ 6) yr, body mass 76.8 ($\pm$ 6.6) kg, stature 178 ($\pm$ 6) cm, and PPO 4.7 ($\pm$ 0.5) W/kg. Participants had been training consistently for >4 yr and prior to entry into the study, trained 7–8 h/wk (range 5–10 h/wk), with four to five training sessions/wk for at least the previous 6 mo. Their training-intensity distribution prior to entering the study was estimated to be 53% zone 1, 38% zone 2, and 9% zone 3, with a training load [intensity zone $\times$ duration (min)] of 750 units. All participants were able to sustain a power output above 240 W for a 40-km TT time prior to entry into the study. Participants were all competitive road cyclists, but some also performed mountain biking within their training. Participants provided written, informed consent to take part in the study, which was approved by the University Ethics Committee in accordance with the Declaration of Helsinki.

Study design. A crossover, within-subject study design was used. All participants were in the study for a period of 29 wk (Fig. 1). This included prescreening and habituation trials in the first 2 wk before commencing a 4-wk controlled detraining period. Participants were then asked not to exercise and to record all of their food and fluid intake for 2 days prior to undertaking a baseline testing week. Following this, the participants entered the training intervention period. Participants ($n = 6$) were assigned to complete POL training first, and $n = 6$ were assigned to complete THR training first. Participants undertook 6 wk of training following either the POL training-intensity distribution (80% low intensity, 0% moderate intensity, 20% high intensity) or a THR training-intensity distribution (57% low intensity, 43% moderate intensity, 0% high intensity). This was followed by a post-training intervention testing week. Participants then completed a second, 4-wk controlled detraining period prior to undertaking the crossover arm of the study, in which they completed a pretraining testing week, 6 wk of training following the alternate training-intensity distribution, and a post-training testing week. The two 6-wk training intervention periods were undertaken over the winter months November–December and January–March.

In the habituation trials, participants undertook at least two 40-km TT test rides on their own bike mounted onto a CompuTrainer ergometer (RacerMate, Seattle, WA). To ensure that we recruited trained cyclists, only riders who completed the 40-km TT with a mean

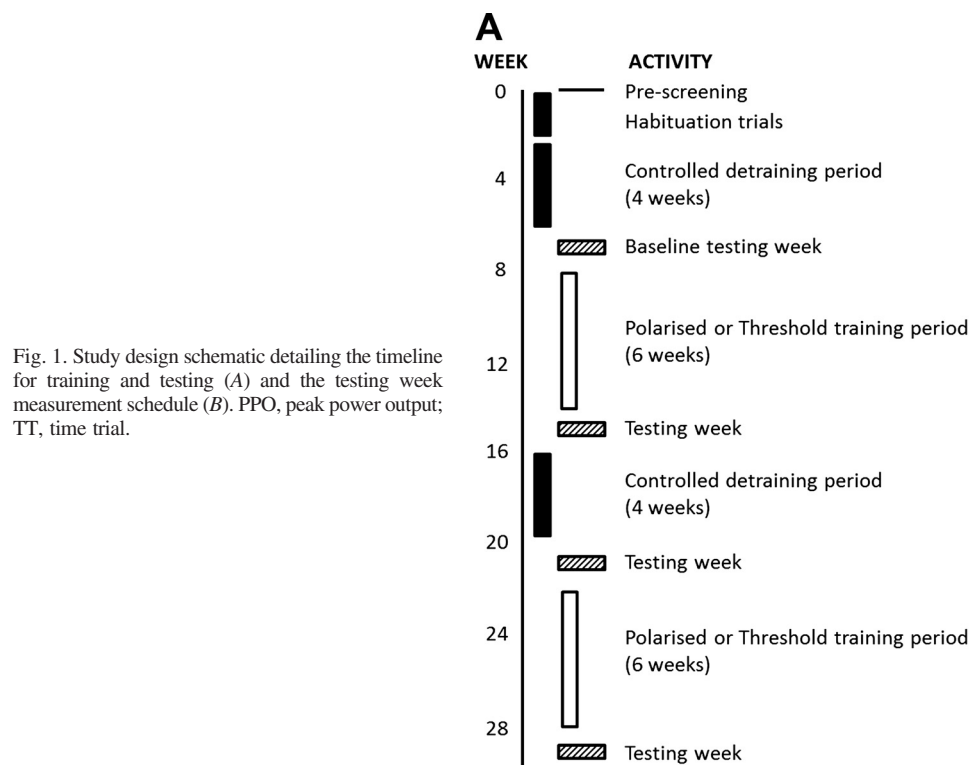


Fig. 1. Study design schematic detailing the timeline for training and testing (A) and the testing week measurement schedule (B). PPO, peak power output; TT, time trial.

B

TESTING WEEK		
DAY	TIME	ACTIVITY
1	0700 - 0900	First void urine sample & skeletal muscle microbiopsy
	1500 - 2000	Lactate threshold and PPO testing followed by 95% PPO exercise capacity testing
4	1500 - 2000	40km TT performance testing

power output of ≥ 240 W were included in the study. During the 4-wk detraining periods, participants were instructed not to include any threshold/tempo rides, interval sessions, or races and to ride exclusively at low-intensity (*zone 1*). Participants completed only 4 h/wk (range 3–5 h/wk) of *zone 1* training during this period. The time at which these detraining periods fell during the study made this possible, as they occurred during October and December–January. This strategy was used to ensure that no specific adaptations from training at moderate or high intensity would be gained in the 4 wk prior to each of the study intervention periods. To determine the effectiveness of the detraining period, we also examined whether PPO, 40-km TT time, mean power output, and high-intensity exercise capacity had all returned to baseline values before beginning the second training intervention.

Physiological adaptation and performance testing. The testing weeks included laboratory-based tests that were conducted on 2 separate days (at least 2 days apart), before and after each training intervention period (Fig. 1). Prior to the first testing session, participants were asked to keep a food (and activity) diary for the 2 days before each of the initial testing sessions, with no exercise on the day preceding any test day. This diary was used to allow them to replicate their diet and activity for the other testing weeks. Likewise, a food diary was kept for the 1st wk of training, and participants attempted to replicate their food intake as closely as possible throughout the training weeks in both interventions. Participants also refrained from caffeine intake in the 3 h before each of the testing sessions. Briefly, on the first visit in each testing week, the participants reported to the laboratory between 0700 and 0900 in a rested, fasted state. A first-pass urine collection was obtained for metabolomics analysis, and a resting skeletal muscle biopsy sample was collected to assess markers of mitochondrial oxidative capacity and lactate transport. The biopsy sample was obtained from the vastus lateralis with the use of a Bard Magnum biopsy system (Bard Peripheral Vascular, Tempe, AZ), as described by Hayot et al. (22), under local anesthesia (2% w/v Lidocaine, 2 ml/subject; B. Braun, Melsungen, Germany). Approximately 20 mg tissue was collected from one to three extractions. The tissue was frozen immediately in liquid nitrogen and stored until later analysis.

Later that day, participants reported to the laboratory for a second time between 1500 and 2000, during which body mass and stature were recorded, followed by an incremental cycle test to determine LTs and PPO. For the incremental test, a CompuTrainer was used in ergometer mode, fitted with the participant's own bike. Following tire-pressure checks (120 psi) and a 10-min light warm-up, the CompuTrainer was calibrated to 3.5 lb, as instructed by the manufacturers for accuracy up to 500 W. The test started at 100 W and increased by 40 W every 3 min until volitional exhaustion, with the cadence remaining self selected but the speed held above 14 mph to ensure accurate measurements of power output. Thirty seconds before the end of each 3-min stage, heart rate (HR; Polar Electro, Kempele, Finland) was recorded, and a capillary blood sample was obtained for blood lactate concentration analysis by microassay (LactatePro LT-1710; ArkRay, Kyoto, Japan). The reliability and validity of this device have been determined previously (42). The LT was determined as the final point before the blood lactate concentration increased distinctly from its resting concentration (1). The LTP was determined as the starting point of accelerated lactate accumulation (1). Each individual's lactate profile was examined independently by two people to identify LT and LTP from the incremental exercise tests. A typical trace would show baseline values for the initial loads, a gradual increase that demarcates the *zone 1* to *zone 2* boundary (LT), and then following several more increments in load, a marked increase is noted that deviates from linearity and reflects the *zone 2* to *zone 3* boundary (LTP). In the present study, the mean ($\pm$ SD) lactate concentration at LT was 2.1 ($\pm$ 0.4) mM and at LTP, was 4.4 ($\pm$ 0.8) mM. This corresponded to an intensity of 64 ($\pm$ 4)% of PPO for LT and 82 ($\pm$ 4)% of PPO for LTP. The PPO was assessed using the following equation: $PPO = W_{\text{final}} + [(t/180) \times 40]$ (31), where W_{final} = the

power output of the final completed stage (W); t = the time spent in the final, uncompleted stage (s); 180 = the duration of each stage (s); and 40 = the increase in power output between each stage (W).

Following the incremental test to exhaustion, the power was decreased to 100 W, and the subject was asked to pedal at a self-selected cadence for 10 min. At 5 min, the CompuTrainer was recalibrated to 3.5 lb. At 10 min, the power output was increased to 95% of PPO, and the subject was requested to maintain a speed above 14 mph until volitional exhaustion to determine high-intensity exercise capacity. The time to fatigue achieved was recorded to the nearest second, along with the peak HR during the test. The 95% PPO load used in the post-training testing was 95% of the pretraining PPO achieved.

On a separate day, at least 2 days following the incremental test to exhaustion, a 40-km TT was performed (Fig. 1). Each participant brought his bike into the laboratory at the same time of day (in the afternoon) and set it up on the CompuTrainer. Following tire checks and a 10-min light warm-up, the CompuTrainer was calibrated to 3.5 lb. Participants were then instructed to complete a 40-km TT as fast as possible. The only data that the participants could see were distance completed. Completion time, HR, and mean power output were recorded.

Training interventions. For 6 wk following the pretraining testing week, participants attended the laboratory on 3 days/wk (Monday, Wednesday, and Friday) for prescribed training sessions. Training intensity was prescribed in relation to the LT and the LTP obtained and used the session-goal approach (46). The aim for POL training was to achieve 80% of training time in *zone 1* and 20% of training time in *zone 3*, with no training time in *zone 2*. The aim for THR was to achieve 55% of training time in *zone 1* and 45% of training time in *zone 2*, with no *zone 3* training time. All laboratory training was completed on the CompuTrainer, which was set up and calibrated as described previously following a 10-min light warm-up.

POL training sessions consisted of six intervals of 4-min duration with 2-min rest periods, similar to the optimal protocol for adaptation identified by the work of Stepto et al. (51). The power output of the six intervals was 5–10% greater than the LTP (i.e., in *zone 3*), with a HR greater than the HR corresponding to the LTP in the incremental test in all cases. During the rest periods, participants either stopped pedaling or pedaled backward, and this remained consistent for every training session. The minimum HR reached during the 2-min recovery period was recorded. A 10-point rating of perceived exertion (RPE), developed by Foster and colleagues (16, 17), was obtained at the end of each training session. If the RPE, mean peak HR, and mean minimum HR were decreasing over two consecutive training sessions, then the power output for the intervals was increased by 5–10 W to maintain a training stimulus. THR sessions included 60 min at a power output half-way between the LT and the LTP (i.e., in *zone 2*). The mean HR for the 60-min session was recorded and RPE obtained at the end of each training session. If the RPE and mean HR were decreasing over two consecutive training sessions, then the power output was increased by 5–10 W to maintain a training stimulus.

The *zone 1* training for both groups consisted of the warm-up and cool-down for the laboratory training sessions (15–20 min/session) combined with low-intensity cycling on the days between the laboratory training sessions. The intensity of the *zone 1* training was controlled with HR, and the mean HR for a *zone 1* session did not exceed the value associated with the LT. Participants were requested to try to maintain their HR at 5 beats/min below the HR, corresponding to the LT at all times during their *zone 1* training sessions. Participants performed two to three *zone 1* training sessions/wk on top of the three laboratory-based training sessions.

Sample analysis. Muscle biopsy samples were prepared for analysis of the maximal activities of citrate synthase (CS) and β -hydroxyacyl-CoA dehydrogenase (β -HAD). Briefly, a small piece of frozen wet muscle (4–5 mg) was removed from the pre- and post-training biopsy samples. The muscle samples were homogenized in 0.1 M KH_2PO_4 and BSA and then subjected to three freeze-thaw cycles. The maximal

activities of CS and β -HAD were then determined on a spectrophotometer (at 37°C) using methods described previously (2, 49) on an ILab Aries analyzer (Instrumentation Laboratory, Milan, Italy). Muscle samples were also used for analysis of monocarboxylate transporters (MCT) 1 and 4 expression. Briefly, 10–15 mg muscle tissue was scissor minced in lysis buffer (50 mM Tris, pH 7.5, 250 mM sucrose, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 1 mM NaVO₄, 50 mM NaF, 0.50% protease inhibitor cocktail) on ice. Samples were shaken for 1 h (800 rpm) at 4°C before centrifuged for 60 min at 12,000 g. The supernatant was removed from the pellet to a fresh tube and used to determine protein concentration using a DC Protein Assay (Bio-Rad Laboratories, Hertfordshire, UK). Equal amounts of protein were then boiled in Laemmli sample buffer (250 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 0.01% bromophenol blue, 5% β -mercaptoethanol), and 7.5 μ g protein from each sample was separated on precast Criterion (Bio-Rad Laboratories) SDS polyacrylamide gels (4–20% gradient gels) for ~90 min at 150 V. Proteins were transferred to a Protran nitrocellulose membrane (Whatman, Dassel, Germany) at 30 V for 2 h. Membranes were blocked in 5% BSA-Tris-buffered saline with 0.1% Tween-20 (TBST) and then incubated overnight at 4°C with the appropriate primary antibody. The primary antibodies were used at the following dilutions: rabbit monoclonal GAPDH 1:5,000 (14C10; Cell Signaling Technology, Danvers, MA), goat polyclonal MCT1 1:1,000 (C-20; Santa Cruz Biotechnology, Santa Cruz, CA), and rabbit polyclonal MCT4 1:1,000 (H-90; Santa Cruz Biotechnology). Following the overnight incubation, the membranes underwent 3 $\times$ 5 min washes in TBST. The membrane was then incubated for 1 h at room temperature with horseradish peroxidase-linked anti-rabbit IgG (1:1,000; 7074; New England Biolabs, Herts, UK) or anti-goat (1:10,000; Abcam, Cambridge, UK), diluted in 5% BSA-TBST. The membrane was then cleared of the antibody using TBST. Antibody binding was detected using enhanced chemiluminescence (GE Healthcare Biosciences, Pittsburgh, PA). Molecular weight was estimated using molecular weight Kaleidoscope Prestained Standards (Bio-Rad Laboratories). In antibody test experiments, GAPDH yielded a single band at the 37-KDa marker, whereas MCT1 and MCT4 antibodies yielded a number of bands with both, displaying a distinct band between 37 KDa and 50 KDa. To improve antibody performance and reduce nonspecific bands and the variability of quantifying different membranes, we carried out the following procedure: prior to transfer, the gels were cut at 25 KDa and 50 KDa molecular weight markers. All of the gel segments for the entire data set were transferred onto a single membrane. This allowed us to visualize more clearly MCT1 and MCT4 as a band running above 37 KDa and below 50 KDa. These membranes were stripped for 30 min at 50°C in stripping buffer (65 mM Tris HCl, 2% SDS vol/vol, 0.8% mercaptoethanol vol/vol) and reblocked, followed by an overnight incubation in anti-GAPDH antibody. Imaging and band quantification were carried out using a bioimaging Gel Doc system (Bio-Rad Laboratories). To determine MCT1/4, the quantities for MCT1/4 were divided by the quantities for GAPDH, and pretraining samples were each then normalized to 1, with post-training samples expressed relative to the respective pretraining data.

Urinary metabolomics analysis was performed using NMR spectroscopy. Urine samples were prepared by the addition of 200 μ l phosphate buffer (0.2 mol/l KH₂PO₄, 0.8 mol/l K₂HPO₄) to 500 μ l urine. Following centrifugation at 8,000 g for 5 min, 10 μ l sodium trimethylsilyl [2,2,3,3-²H₄] propionate (TSP) and 50 μ l D₂O were added to 550 μ l of the supernatant. Spectra were acquired on a 600-MHz Varian NMR spectrometer using the first increment of a nuclear Overhauser effect spectroscopy pulse sequence at 25°C. Spectra were acquired with 16 K data points and 128 scans over a spectral width of 9 kHz. Water suppression was achieved during the relaxation delay (1 s) and the mixing time (200 ms). All ¹H NMR urine spectra were referenced to TSP at 0.0 ppm and processed manually with Chenomx (version 6) using a line broadening of 0.2

Hz. The spectra were integrated into bins consisting of spectral regions of 0.04 ppm, using Chenomx (version 6). The water region (4.0–6.0 ppm) was excluded, and the data were normalized to the sum of the spectral integral.

Statistical analyses. Statistical analysis was performed using SPSS version 18 software (IBM, Armonk, NY). A fully repeated measures ANOVA (2 $\times$ 2) compared the performance/physiological adaptation measures between training-intensity distribution models (POL and THR) and over time (pre- to post-training). Main effects among training-intensity distribution models, over time, and any interaction between these and the performance/physiological adaptation measures were reported. Post hoc analysis was undertaken where significant main effects were obtained by using paired Student's *t*-tests and two-tailed values of *P*, with the Bonferroni method of adjustment to prevent type I error. Paired Student's *t*-tests using two-tailed values of *P* were also used to compare training variables at baseline between POL and THR. The urinary metabolomics data were analyzed using a multivariate data analysis performed using SIMCA-P+ software (version 11.0; Umetrics, Umeå, Sweden). Data sets were scaled using unit variance scaling. Principal component analysis (PCA) was applied to data sets to explore any trends or outliers in the data. To probe the effects of training-intensity distribution, the data were analyzed using multilevel partial least squares-discriminant analysis (PLS-DA), as used previously in metabolomics studies (54).

Statistical significance was accepted at *P* < 0.05. All data in the text and tables are expressed as mean ($\pm$ SD) and in figures as mean ($\pm$ SE). Effect sizes for the key performance/physiological adaptation measures were calculated from the mean difference (pre to post), divided by the SD of the baseline measure. These values were judged using the descriptors suggested by Cohen (7). Effect sizes were included to highlight the magnitude of the performance/physiological adaptation changes.

RESULTS

One participant did not complete the study due to injury. Training adherence for the 11 remaining participants was 96% and 97% for POL and THR, respectively. The total training volume was significantly higher for THR than POL (Table 1; *P* < 0.05). This was due to the nature of the study design in which we attempted to match the volume of training in zone 1 between POL and THR training models [mean ($\pm$ SD) zone 1 time was 313 ($\pm$ 65) and 283 ($\pm$ 76) min/wk for POL and THR, respectively; no significant difference]. The percentage of time spent in each training-intensity zone (zone1:zone2:zone3) was the intended 80:0:20 distribution for POL and was close to intended at 57:43:0 distribution for THR (Table 1). Body mass was not different between training periods and did not change from pre- to post-training in either POL (76.5 $\pm$ 6.3–76.6 $\pm$ 6.2 kg) or THR (77.3 $\pm$ 6.7–76.5 $\pm$ 6.0 kg) training periods.

Table 1. Mean ($\pm$ SD) details of the total training time completed/wk for the polarized (POL)- and threshold (THR)-training models, the training load (intensity zone $\times$ duration, min), and the proportion of training time spent in zone 1, zone 2, and zone 3

	Units	POL	THR
Total training time	min/wk	381 ($\pm$ 85)	458 ($\pm$ 120)*
Training load	intensity zone $\times$ duration	517 ($\pm$ 90)	633 ($\pm$ 119)*
Zone 1	% of training time	80 ($\pm$ 4)	57 ($\pm$ 10)*
Zone 2	% of training time	0 ($\pm$ 0)	43 ($\pm$ 10)*
Zone 3	% of training time	20 ($\pm$ 4)	0 ($\pm$ 0)*

*Difference between POL and THR (*P* < 0.05).

Table 2. Power output, heart rate (HR), and rating of perceived exertion (RPE) sustained during the laboratory training sessions for the polarized (6 × 4 min, zone 3 intensity bouts) and threshold (60-min constant zone 2 intensity bouts) training

Variable	Training Model	1	2	3	4	5	6
Power output, W	POL	319 (±33)	321 (±34)	328 (±35)*†	331 (±37)*†	337 (±35)*†‡§	340 (±34)*†‡§
Peak HR, beats/min		173 (±10)	172 (±9)	173 (±10)	173 (±9)	172 (±9)	171 (±9)
Recovery HR, beats/min		111 (±14)	111 (±10)	109 (±15)	109 (±12)	108 (±13)	108 (±14)
RPE, 0–10		7 (±1)	7 (±1)	8 (±1)	8 (±1)	8 (±1)	7 (±1)
Power output, W	THR	266 (±31)	267 (±33)	277 (±34)*†	284 (±33)*†‡	288 (±33)*†‡§	290 (±32)*†‡§
HR, beats/min		158 (±12)	155 (±10)	156 (±9)	157 (±9)	159 (±8)	159 (±9)
RPE, 0–10		5 (±1)	5 (±1)	6 (±1)	6 (±1)	6 (±1)	6 (±1)

Values are mean (±SD) from 3 laboratory training sessions in each week during the study. All values are different between POL and THR ($P < 0.05$). *Significant difference from week 1, †from week 2, ‡from week 3, and §from week 4 within each training model ($P < 0.01$).

There was a main effect over time for the mean power output sustained during each of the 4-min intervals in the POL training sessions ($P < 0.05$) to maintain the training stimulus, with a significant increase from week 1 observed by week 3 (Table 2). Due to the increase in target load, there were no differences over time for the peak HR reached during the sessions, the mean minimum HR following the 2-min recoveries, or the RPE rating of the session over the 6 wk (Table 2). There was also a main effect over time for the power output sustained during the 60-min threshold exercise-training sessions ($P < 0.05$) to maintain the training stimulus, with an increase from week 1 observed by week 3 (Table 2). Due to the increase in target load, there were no differences over time for the mean HR sustained during the 60-min ride or the RPE rating of the session over the 6 wk (Table 2).

Endurance performance and physiological adaptation. There was a main effect over time for LT, LTP, and PPO ($P < 0.05$; Fig. 2). There was also a significant interaction ($P < 0.05$) with the training-intensity distribution model for LT and PPO. A significant increase was observed for LT power and PPO from pre- to post-training in POL [18 (±18) W for LT and 27 (±18) W for PPO; both $P < 0.05$], but this was not observed with the THR training model [4 (±31) W for LT power and 9 (±17) W for PPO; both not significant]. The effect sizes for the changes in LT and PPO were both classed as moderate for the POL model but were classed as trivial and small for the THR model (Table 3). The percentage change in LT and PPO from pre- to post-training was higher in POL than THR [9 (±9)% POL vs.

2 (±14)% THR for LT and 8 (±5)% POL vs. 3 (±4)% THR for PPO; both $P < 0.05$].

There was a main effect over time for 40-km TT mean power output ($P < 0.05$; Fig. 3). The mean power output was higher from pre- to post-training with both POL and THR training. The absolute change (Fig. 3) and percentage change in the mean power output from pre- to post-training were higher in POL than THR [8 (±8)% and 4 (±6)%, respectively] but did not reach statistical significance. The time to complete the 40-km TT improved by 2.3 (±2.2) min vs. 0.4 (±2.9) min following POL vs. THR training, respectively. The effect size was deemed moderate for POL and small for THR (Table 3).

There was also a main effect over time for the high-intensity exercise capacity at 95% of pretraining PPO ($P < 0.05$; Fig. 3), with increases from pre- to post-training for both POL and THR models ($P < 0.05$). There was also an interaction effect ($P < 0.05$) with a significantly greater percentage increase from pre- to post-training in POL [85 (±43)%] compared with THR [37 (±47)%].

Detraining appeared to be effective, with initial PPO before the first and second training interventions not significantly ($P = 0.94$) different [359 (±31) W and 359 (±39) W, respectively]. The same was true for high-intensity exercise capacity, which was not different ($P = 0.46$) before the first and second training interventions [286 (±60) s and 304 (±45) s, respectively]. The 40-km TT time [65 (±5) min vs. 63 (±3) min] and mean power output sustained during the TT [281 (±37) W vs.

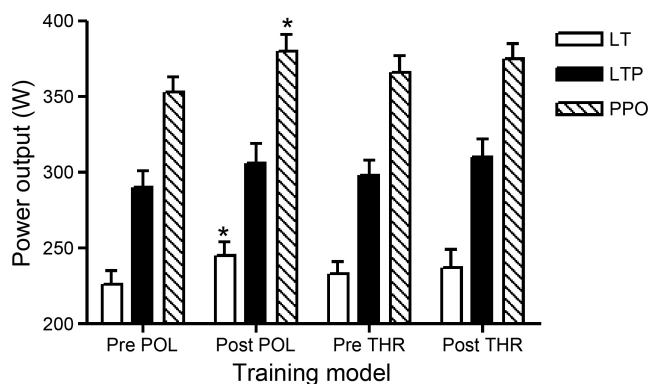


Fig. 2. Mean (±SE) power output corresponding to the lactate threshold (LT), lactate turn point (LTP), and PPO before (Pre) and following (Post) both of the 6-wk training interventions. POL, polarized training model; THR, threshold training model. *Significantly different from pre within a specific training model ($P < 0.05$).

Table 3. Mean (±SD) percentage change (Δ , %) and effect sizes for the key performance and adaptation measures assessed before and after 6 wk of polarized and threshold training interventions

Training Model	Measure	Δ , %	Effect Size	Descriptor*
POL	40-km TT MPO, W	8 (±8)	0.57	Moderate
	LT, W	9 (±9)†	0.59	Moderate
	LTP, W	6 (±10)	0.40	Small
	PPO, W	8 (±5)†	0.77	Moderate
	95% exercise capacity, s	85 (±43)†	2.44	Large
THR	40-km TT MPO, W	4 (±6)	0.35	Small
	LT, W	2 (±14)	0.11	Trivial
	LTP, W	4 (±7)	0.34	Small
	PPO, W	3 (±4)	0.26	Small
	95% exercise capacity, s	37 (±45)	0.99	Large

TT, time trial; MPO, mean power output; LT, lactate threshold; LTP, lactate turnpoint; PPO, peak power output; 95% exercise capacity, time to exhaustion at 95% of pretraining PPO. *Cohen (7); †significant difference between POL- and THR-training models ($P < 0.05$).

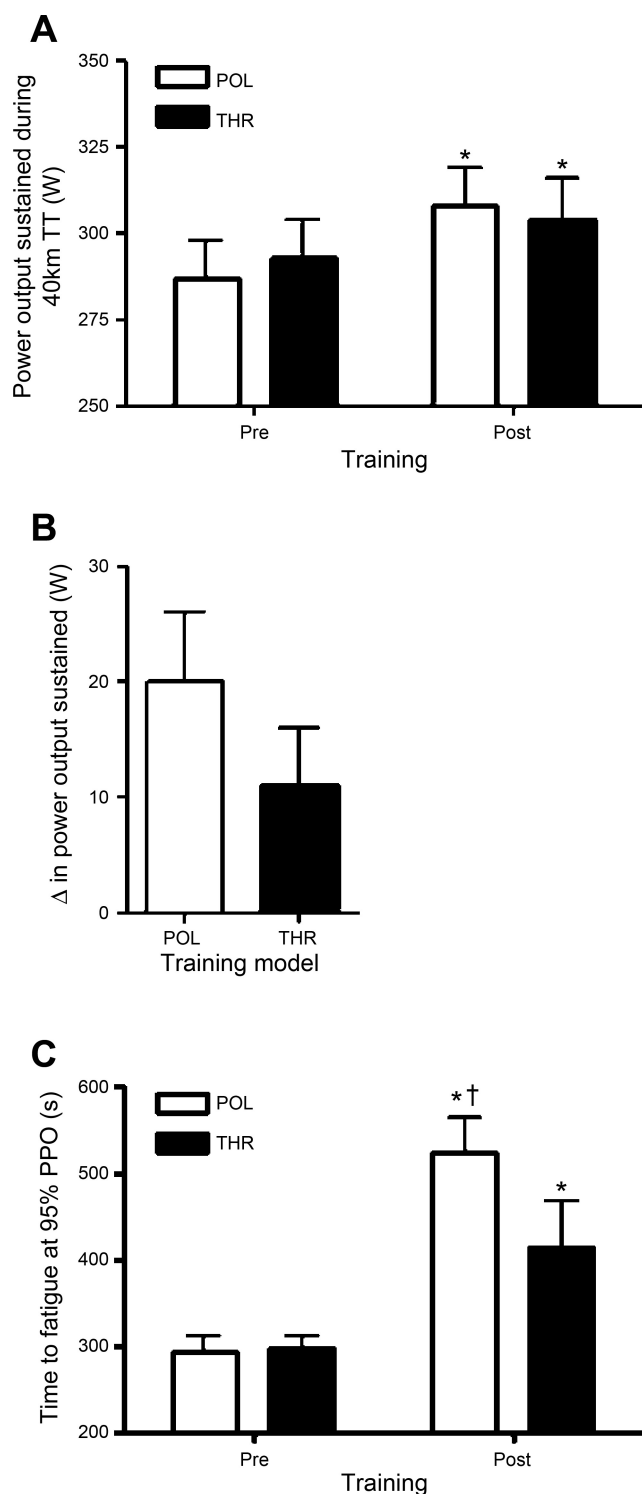


Fig. 3. Mean ($\pm$ SE) 40-km TT mean power output assessed before (Pre) and following (Post) both of the 6-wk training interventions (A), absolute change (Δ) in mean power output sustained during the 40-km TT following the training interventions (B), and time to fatigue at 95% of baseline PPO assessed before and following both of the 6-wk training interventions (C). *Different from pre within a training model ($P < 0.05$); †significant difference between training models.

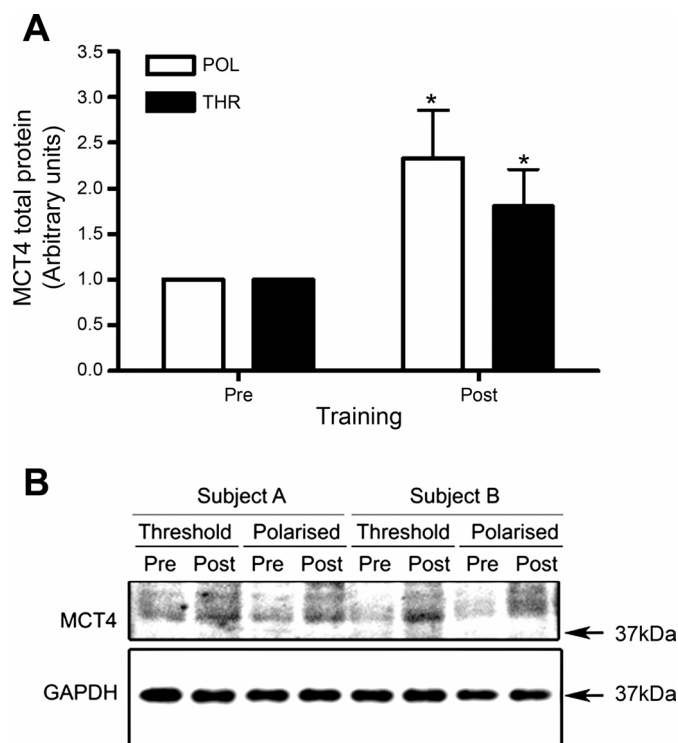


Fig. 4. Mean ($\pm$ SE) skeletal muscle change in total protein content from pre- to post-POL and -THR training interventions for monocarboxylate transporter protein 4 (MCT4; A) and representative blots from 2 participants (B). *Significantly different from Pre within a specific training model ($P < 0.05$).

289 ($\pm$ 36) W] both followed this same pattern by returning toward initial values ($P = 0.06$). Training load [intensity zone $\times$ duration (min)] dropped substantially during the detraining period. The training load was reduced to 38–46% of that sustained during THR and POL, respectively.

Skeletal muscle analysis. There were no main effects over time or with the training-intensity distribution model for the maximal activities of the skeletal muscle oxidative enzymes studied. The maximal activity of CS from pre- to post-training with POL and THR was 47 ($\pm$ 6)–48 ($\pm$ 4) mmol·kg wet wt⁻¹·min⁻¹ and 47 ($\pm$ 5)–49 ($\pm$ 3) mmol·kg wet wt⁻¹·min⁻¹, respectively. The maximal activity of β -HAD from pre- to post-training was 15 ($\pm$ 2)–15 ($\pm$ 2) mmol·kg wet wt⁻¹·min⁻¹ and 15 ($\pm$ 2)–15 ($\pm$ 1) mmol·kg wet wt⁻¹·min⁻¹ with POL and THR, respectively. MCT1 expression was unchanged over either of the exercise-training periods [12% ($\pm$ 13%) increase for pre- to post-training with POL and 10% ($\pm$ 13%) increase for pre- to post-training with THR]. However, MCT4 expression was increased over both training periods (Fig. 4). There was a 133% ($\pm$ 56%) increase in MCT4 total protein with POL training and an 80% ($\pm$ 41%) increase in MCT4 total protein with THR training. There was no interaction between time and training model for MCT1 or MCT4 protein expression.

Urinary metabolomics. Initial PCA analysis was performed and did not reveal any separation according to training-intensity distribution. A significant multilevel PLS-DA model was obtained for the THR training period but not for the POL training ($P < 0.05$). The NMR regions changing, following the THR training period, were identified using the rank product (RP) plot (Fig. 5). The RP plot shows the discriminating

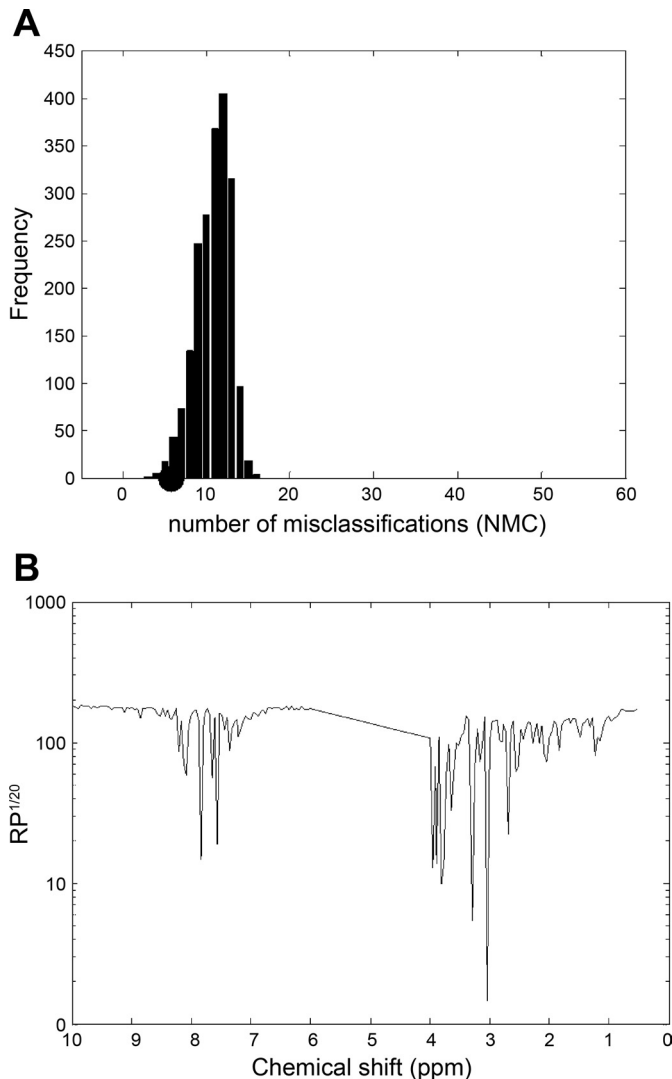


Fig. 5. Mean cross-model validation (CMV) prediction error (A). The dot indicates the CMV prediction error estimated in terms of number of misclassifications. The variable ranks product (RP) plot for the model is also shown (B).

metabolites for the model. The metabolites responsible were identified as hippuric acid, creatinine, dimethylamine, 3-methylxanthine, hypoxanthine, and an unidentified peak at 3.28 ppm. The direction of change for these metabolites was identified as hippuric acid decreased post-THR, creatinine increased post-THR, dimethylamine increased post-THR, 3-methylxanthine increased post-THR, and hypoxanthine decreased post-THR.

DISCUSSION

Endurance athletes have been repeatedly demonstrated to undertake a high training volume with ~80% of training time at low intensities (*zone 1*) and ~20% of training time at higher intensities (*zones 2 and 3*, combined) (19, 35, 36, 43, 45). Previous studies have described a performance benefit from adding high-intensity training bouts into the overall training of endurance athletes (33, 51, 57, 58). However, the present study demonstrates for the first time in a randomized crossover study design that adopting this type of training-intensity distribution leads to greater adaptations over 6 wk compared with a

training-intensity distribution focused more around moderate (threshold) intensities (57% in *zone 1*; 43% in *zone 2*). Notably, this outcome occurs despite a greater total training volume with the THR training model and occurs in already well-trained cyclists. In particular, LT, PPO, and exercise capacity at 95% of pretraining PPO all improved to a greater extent with POL compared with THR training. Although there was no statistically significant difference between POL and THR for the improvement in 40-km TT mean power output, the effect size was larger for POL, and the magnitude of change was twice that observed following THR training. The greater effect sizes for all of the key performance and adaptation markers with POL compared with THR training provide a strong indicator that POL training is more optimal for short-term training adaptations to occur. The muscle enzyme activity analysis and MCT1/4 expression suggest that these performance adaptations are independent of detectable differences in mitochondrial oxidative capacity or differences in lactate transport/oxidation in skeletal muscle between training models. However, the metabolomics analysis reveals that some markers of cellular energy stress were modified with THR but not with POL. Collectively, these data provide some new insights into understanding training stress and optimal intensity distribution for adaptation in already well-trained athletes.

It has been suggested previously that endurance athletes might not achieve optimal gains in performance and/or physiological adaptation by doing too much moderate-intensity training in *zone 2* (12, 27, 34). Previous work has also shown that a group of elite runners who trained more at an intensity corresponding to the LT (*zone 2*) had a lower performance level than a group of elite runners that trained less in *zone 2* and more in *zone 3* (3). The evidence from the present study and these previous studies suggests that a critical component for promoting adaptation is the incorporation of high-intensity interval training (*zone 3*) sessions and reduction of moderate-intensity (*zone 2*) sessions, while maintaining the volume of low-intensity (*zone 1*) sessions. Whereas we appreciate that trained athletes will incorporate all three intensity zones into their training schedules and competitions, our aim was to determine the impact of high-intensity interval work vs. moderate-intensity continuous threshold training sessions on adaptation. It would seem that reducing the emphasis on moderate-intensity threshold work in place of high-intensity interval work promotes greater adaptation. This may be particularly true for our cyclists who had not followed a POL training model prior to entry into our study. The precise mechanisms for these beneficial effects in already well-trained individuals are not fully understood, but we do know that exercise intensity is a key driver for adaptation from several short-duration training studies (38, 51, 52). Recently, there has been some debate about the benefits of polarized training, suggesting that it helps to reduce fatigue and may be a more optimal stimulus for adaptation based on our genetic makeup and activity profiles of ancestors (4).

Higher training intensity (*zone 3* vs. *zone 2*) should cause a greater increase in the activation of adenosine monophosphate-activated protein kinase (AMPK), as has been reported in previous studies (5, 60). Indeed, in a group of well-trained cyclists, a high-intensity training session involving 8 × 5 min at 85% peak oxygen consumption caused an increase in AMPK activity and phosphorylation (6). AMPK-signaling mecha-

nisms are linked to the initiation of mitochondrial biogenesis through the regulation of peroxisome proliferator receptor- γ coactivator-1 α expression and activity (41). In the present study, however, it seems that the differences in endurance performance and physiological adaptation between POL and THR were not due to differences in mitochondrial oxidative capacity, as there were no changes in the maximal activity of CS or β -HAD between training models or in response to the training. The absence of a detectable change in mitochondrial oxidative capacity is likely due to the skeletal muscle of the cyclists already having a high mitochondrial oxidative capacity at the start of the training. This is demonstrated in the absolute magnitude of enzyme activities and the performance measures attained. Any further increase in muscle oxidative capacity in already well-trained skeletal muscle is likely to be small and may be too small to be detectable following a short-term training intervention (30). Indeed, following high-volume, high-intensity training in well-trained athletes, there was no change in the maximal activity of CS (14, 58) and β -HAD (58), despite improvements in endurance performance. In contrast, studies using moderately trained individuals and a similar interval-training program component to that in the POL model in the present study have found large increases in the maximal activities of CS and β -HAD of 20–30% (20, 38, 52). It is also possible that an insufficient, additional training stimulus could explain the lack of mitochondrial oxidative capacity response in athletes compared with the usually large improvements noted in studies on moderately trained individuals. However, it seems likely that there may also be a ceiling for adaptation in mitochondrial oxidative capacity in already well-trained skeletal muscle. Therefore, in the present study, it would appear that the improvements in physiological performance parameters during exercise are independent of detectable changes in mitochondrial oxidative capacity.

The significant increase in LT and greater change in time to exhaustion (TTE) at 95% of pretraining PPO following POL compared with THR training most likely reflects adaptations induced by the higher-intensity interval exercise. Since the absolute training volume at low intensity (*zone 1*) was closely matched, these differences in adaptation must come down to the training time spent in *zone 2* or *zone 3*, with *zone 3* proving more effective. It has been reported that the lactate transport capacity of skeletal muscle is increased by training and that MCT1 and MCT4 content is increased following high-intensity knee extensor exercise training over 8 wk (39). These prior data show that intense exercise influences lactate/ H^+ transporter expression and could explain the present LT and TTE data. Interestingly, changes in intracellular lactate shuttling between *type II* and *type I* fibers and increases in capacity for lactate oxidation through upregulation of mitochondrial lactate dehydrogenase and mitochondrial MCT1 (as part of a lactate oxidation complex) (21) could provide an explanation for our observations. However, in the present study, neither the high-intensity interval exercise in the POL model nor the continuous moderate-intensity session in the THR model was effective at inducing changes in total MCT1 content. Since MCT1 occupies both mitochondrial and sarcolemmal domains in skeletal muscle, the lack of any change in whole muscle MCT1 content potentially mirrors and supports the lack of change in mitochondrial oxidative enzyme activity, due to an already large training base in our participants. The lack of change in MCT1

adds support to the notion that already well-trained cyclists, with high preintervention mitochondrial oxidative capacity, will have little capacity for further mitochondrial adaptation. Furthermore, with no evidence for mitochondrial adaptation, it could be suggested that any change observed in MCT1 content would then reflect sarcolemmal MCT1. On this basis, our data also highlight that there are no detectable sarcolemmal changes in MCT1 in already well-trained cyclists undertaking these interventions. However, further work is required to investigate specific sarcolemmal and mitochondrial MCT1 changes with training interventions to provide more insight into the precise mechanisms underpinning the greater adaptations in LT and high-intensity exercise capacity observed with POL vs. THR.

The increase in MCT4 content in both training models is also interesting. This observation suggests that the continuous moderate-intensity work in the THR model and the high-intensity work in the POL intervention both provide a good stimulus to MCT4 expression. MCT4 occupies a sarcolemmal domain only and is thought to largely contribute to extrusion of H^+ and lactate from the cell cytosol. An improved maintenance of intracellular pH and lactate concentration has been considered a factor that could delay the development of fatigue during high-intensity tasks and as such, may explain our observations. However, understanding MCT4 adaptations to exercise training is still incomplete. It has been suggested that increases in MCT4 should occur in line with increases in MCT1 expression, but this has not been observed in the present work. It is notable that a recent review highlighted that the reported responses of MCT1 and MCT4 to exercise may be influenced by the timing of the post-training biopsies (53). In the present study, the biopsies were obtained at least 24 h following the last training session—a time at which observed changes in MCT4 may be high, and changes in MCT1 may be low (53). Therefore, further work remains to be done in already well-trained individuals to understand the relationships among training intensity, timing of tissue sampling, MCT expression, and adaptations in high-intensity exercise capacity.

Other contributing factors to changes in LT and TTE at 95% of pretraining PPO must also be considered and could include greater increases in buffering capacity, improved capillarity, or other systemic cardiovascular adaptations that have all been reported to be increased to a greater extent after high-intensity exercise training (10, 24, 59) and are also related to muscular exercise performance/capacity (25). Alternatively, as exercise intensity increases, there is also a greater recruitment of fast-twitch muscle fibers (9). Adaptations in muscle are observed to be greatest in those muscle fibers that are activated directly during training (9). It has also been suggested that fast-twitch muscle fibers become more fatigue resistant following high-intensity training. These observations could partly explain the greater improvements in the PPO and TTE at 95% of pretraining PPO in POL compared with THR.

The effectiveness of a training-intensity distribution containing ~80% of total training time in *zone 1* and ~20% in *zone 3*, as used for POL in the present study, has been suggested to be due not only to the intensity-specific adaptations but also to enhanced recovery (47). Therefore, the recovery between training sessions could partly explain the effectiveness of POL compared with THR. It has been reported that the acute recovery from a training session in *zone 1* is faster than following a training session in *zone 2*, yet the recovery following a training session in *zone 3* is no

different than following a session in *zone 2* (48). If *zone 3* training leads to larger physiological adaptations compared with *zone 2* yet with similar recovery, then this could be considered a more effective training strategy. Moreover, since recovery is greater from *zone 1* than *zone 2* sessions, it has been recommended to supplement *zone 3* training with training in *zone 1* (48). In the present study, improvements in the 40-km TT and the TTE for both POL and THR suggest that over-reaching did not take place during either of the training interventions. This could be interpreted as recovery being a minor issue. However, POL training appears to provide a stronger stimulus for physiological adaptation and improvement in 40-km TT performance, as well as produce larger gains in high-intensity exercise capacity. Therefore, enhanced recovery cannot be ruled out as a potential factor contributing to the greater adaptations.

The urinary metabolomics data are interesting since a significant model was only observed from pre- to post-training for THR. Of course, it could be argued that the metabolites of interest may reflect dietary influences (caffeine intake, phytochemical intake, protein intake), as has been reported in previous metabolomics and nutrition studies (54, 56) and highlighted in a review by Gibney et al. (18). However, dietary intake was controlled with participants replicating their food intake for 2 days prior to the morning first-pass urine sample collections. Participants did not exercise on the day before collection of the samples, and the THR and POL training interventions were administered in a randomized crossover fashion. Therefore, it would seem that dietary intake would be an unlikely key factor here. Alternatively, the metabolites of interest could collectively suggest differences in cellular metabolic/energy stress induced by the THR training. Greater creatinine excretion would normally reflect greater plasma creatinine degradation, act as a marker of glomerular filtration rate, or mirror lean body mass in 24-h urine collections (15). In morning first-void urine, it likely reflects changes in hydration status or possibly reflects energy availability (55). In the absence of dietary influences, urinary dimethylamine is thought to reflect intermediary metabolism (37), whereas hypoxanthine reflects purine nucleotide degradation, which tends to be acutely lower if high-intensity sprint exercise is not undertaken (50). Urinary 3-methylxanthine can be produced by demethylation of theophylline in the presence of oxidizing radicals (44), and increased urinary excretion of this metabolite could therefore represent greater overall oxidative stress from the THR training period. Changes in hippurate excretion are typically associated with gut microflora (62), and activities of gut microflora may play an important role in energy metabolism and/or immune function of the whole organism (28).

Whereas the metabolomics profile change following THR but not POL cannot be fully explained, it may provide some insight into the overall cellular metabolic/energetic stress experienced with the THR training model. Greater evidence of cellular metabolic/energy stress with the THR model would support the notion of longer recovery times from threshold training sessions or may just reflect the higher training load. Either way, this greater stress was not associated with greater adaptation, which may suggest a maladaptive response to the THR training. These new insights provide some preliminary evidence that metabolomics may be useful in tracking and identifying novel markers related to training stress, adaptation, and recovery. Clearly, the sample size in the present study is one limitation for the metabolomics analysis, but future work

in larger-scale studies may help to verify the usefulness of metabolomics profiling of training stress.

Conclusions

The present study therefore confirms the hypothesis that a polarized training-intensity distribution model is an effective strategy in already well-trained endurance athletes. A polarized training model is recommended for trained cyclists wishing to maximally improve performance and physiological adaptation over a short-term training period, particularly if they are currently following a threshold training distribution model. There is, however, much still to be understood regarding the impact of endurance-training periods containing different training-intensity distributions in endurance athletes and the mechanisms responsible for these effects. Therefore, this is a fruitful area for future research that can contribute not only to the optimization of endurance-training programs for athletes but also to the understanding of optimal ways to promote physiological adaptations to exercise in the wider population.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: C.M.N., A.M.H., G.De V., and S.D.R.G. conception and design of research; C.M.N., A.M.H., G.De V., and S.D.R.G. performed experiments; C.M.N., L.B., A.O., D.L.H., and S.D.R.G. analyzed data; C.M.N., A.M.H., L.B., A.O., G.De V., and S.D.R.G. interpreted results of experiments; C.M.N., L.B., A.O., D.L.H., and S.D.R.G. prepared figures; C.M.N. and S.D.R.G. drafted manuscript; C.M.N., A.M.H., D.L.H., G.De V., and S.D.R.G. edited and revised manuscript; C.M.N., A.M.H., L.B., A.O., D.L.H., G.De V., and S.D.R.G. approved final version of manuscript.

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