

Determinants of time trial performance and maximal incremental exercise in highly trained endurance athletes

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¹Institute of Veterinary Physiology, University of Zurich; ²Center for Integrative Human Physiology (ZIHP); and ³Institute of Physiology, University of Zurich, Zurich, Switzerland; ⁴Institute of Pharmacology and Neuroscience, University of Copenhagen, Copenhagen, Denmark; ⁵Department of Health and Human Performance, Universidad Politécnica de Madrid, Madrid, Spain; ⁶Department of Transplant Surgery, D. Swarovski Research Laboratory, Innsbruck Medical University, Innsbruck, Austria; ⁷Department of Exercise and Sport Sciences, University of Copenhagen, Copenhagen, Denmark; and ⁸Ecole Nationale des Sports de Montagne, Chamonix, France

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Jacobs RA, Rasmussen P, Siebenmann C, Díaz V, Gassmann M, Pesta D, Gnaiger E, Nordsborg NB, Robach P, Lundby C. Determinants of time trial performance and maximal incremental exercise in highly trained endurance athletes. *J Appl Physiol* 111: 1422–1430, 2011. First published September 1, 2011; doi:10.1152/jappphysiol.00625.2011.—Human endurance performance can be predicted from maximal oxygen consumption ($\dot{V}O_{2\max}$), lactate threshold, and exercise efficiency. These physiological parameters, however, are not wholly exclusive from one another, and their interplay is complex. Accordingly, we sought to identify more specific measurements explaining the range of performance among athletes. Out of 150 separate variables we identified 10 principal factors responsible for hematological, cardiovascular, respiratory, musculoskeletal, and neurological variation in 16 highly trained cyclists. These principal factors were then correlated with a 26-km time trial and test of maximal incremental power output. Average power output during the 26-km time trial was attributed to, in order of importance, oxidative phosphorylation capacity of the vastus lateralis muscle ($P = 0.0005$), steady-state submaximal blood lactate concentrations ($P = 0.0017$), and maximal leg oxygenation ($sO_{2\text{LEG}}$) ($P = 0.0295$), accounting for 78% of the variation in time trial performance. Variability in maximal power output, on the other hand, was attributed to total body hemoglobin mass (Hb_{mass} ; $P = 0.0038$), $\dot{V}O_{2\max}$ ($P = 0.0213$), and $sO_{2\text{LEG}}$ ($P = 0.0463$). In conclusion, 1) skeletal muscle oxidative capacity is the primary predictor of time trial performance in highly trained cyclists; 2) the strongest predictor for maximal incremental power output is Hb_{mass} ; and 3) overall exercise performance (time trial performance + maximal incremental power output) correlates most strongly to measures regarding the capability for oxygen transport, high $\dot{V}O_{2\max}$ and Hb_{mass} , in addition to measures of oxygen utilization, maximal oxidative phosphorylation, and electron transport system capacities in the skeletal muscle.

mitochondria; oxidative phosphorylation; oxygen transport and utilization; skeletal muscle; hemoglobin

THE PRODUCT of an athlete's maximal oxygen consumption ($\dot{V}O_{2\max}$) \times lactate threshold \times exercise efficiency can predict endurance performance (4, 44–46). This general calculation of performance is likely appropriate because it broadly accounts for most physiological parameters involved in exercise. The purpose of this study is therefore to isolate the most dominant physiological variables of performance and identify the stron-

gest determinant(s) of exercise performance in highly trained endurance athletes.

An individual's $\dot{V}O_{2\max}$ is limited primarily by cardiac output, locomotor muscle blood flow, and oxygen-carrying capacity of the blood (1, 47, 52) while also expressing a strong linear correlation with mitochondrial volume density (85). The constraint of oxygen consumption has been recognized to limit exercise performance for nearly a century (20, 33), and a large $\dot{V}O_{2\max}$ is a prerequisite for high endurance capability (72, 74, 78). The ability to maintain a given workload with lower blood lactate concentrations, however, correlates more reliably to endurance performance than $\dot{V}O_{2\max}$ among groups of trained individuals (17, 22, 70) and is said to be the strongest broad-spectrum predictor of exercise performance in highly trained endurance athletes (17, 55). The lactate threshold is a theoretical workload facilitating an accelerated accumulation of blood lactate attendant to a disproportionate rise in glycolytic flux and lactate appearance over oxidative phosphorylation and lactate disposal, respectively (9, 24). The physiological determinants of maintaining relatively low concentrations of lactate are believed to parallel the mitochondrial capacity of the skeletal muscle (35, 84). Additionally, skeletal muscle oxidative capacity, once thought to be indicated by percentage of type 1 (oxidative) muscle fibers (2), correlates strongly with exercise efficiency (18), which also accounts for a significant variation in exercise performance among highly trained athletes (14, 58). Oxidative phosphorylation capacity of the skeletal muscle appears central to all three physiological components used to predict endurance performance ($\dot{V}O_{2\max}$, lactate threshold, and exercise efficiency) although its relation to exercise performance in a homogeneous group of trained athletes has never been directly demonstrated.

Accordingly, during a recent study involving 16 highly trained endurance cyclists (Siebenmann C, Robach P, Jacobs RA, Rasmussen P, Nordsborg N, Díaz V, Christ A, Olsen NV, Maggiorini M, Lundby C, unpublished observations), we acquired a considerable physiological data set and correlated these to time trial performance and maximal incremental exercise capacity. As oxidative capacity of the skeletal muscle appears to have the most common interrelation between $\dot{V}O_{2\max}$, lactate threshold, and exercise efficiency we hypothesize that maximal oxidative phosphorylation capacity of the skeletal muscle is the strongest determinant of time trial performance in highly trained athletes.

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METHODS

Volunteers

Sixteen highly trained endurance athletes (15 men, 1 woman, age 29 ± 6 yr, height 179 ± 8 cm, body weight 69 ± 9 kg) from various countries in North America and Europe were volunteers in the current study. All of them regularly participated in endurance competitions on at least the national level in disciplines related to cycling, i.e., road cycling, triathlon, cycle cross, and/or mountain bike. All volunteers gave written informed consent to participate in the study. The study was approved by the Ethics Committee of Zürich (2010–066/0) and Vaud (215/10) (Switzerland), and conformed to the Declaration of Helsinki.

Experimental Protocols

All the experimental procedures of the study were performed at the hospital La Vallée (Le Sentier, Switzerland). At least 24 h was given to all subjects in between separate exercise tests.

Maximal incremental exercise capacity. This measure of performance was tested on an electronically braked bicycle-ergometer in an upright position (Monark 839E, Varberg, Sweden). The exercise protocol started with a warm-up period of 5 min at a workload of 150 W followed by 5 min at 200 W except for the female athlete who warmed up at 100 and 150 W. Thereafter, the workload was increased by 25 W/min until failure. During the last minutes of the test, volunteers were vigorously encouraged to perform to complete exhaustion. Volunteers wore a face mask covering mouth and nose for breath collection, and O₂ and CO₂ concentration in the expired gas were continuously measured and monitored as breath-by-breath values. The gas analyzers and the flowmeter of the applied spirometer (Quark, Cosmed, Rome, Italy) were calibrated prior to each test. After the test breath-by-breath values were visually controlled and averaged over 30 s. The highest average value was determined for $\dot{V}O_{2\max}$, and all the other parameters were selected at the same time except for maximal workload (W_{\max}), which was calculated as $W_{\max} = W_{\text{compl}} + 25 \times (t/60)$, with W_{compl} being the last completed workload and t the number of seconds in the not-completed workload.

Time trial performance. To evaluate exercise performance in a scenario similar to competitions, volunteers performed a time trial using their own personal bike mounted on an electrically braked cycle trainer (Fortius Virtual Reality Trainer, Tacx, Rotterdam, Netherlands). The combination with a commercially available software allowed for the simulation of a predefined route on a portable computer. We selected a final section of the Milan-San Remo race with a length of 26.15 km. This route consists of a first flat part (4.8 km, average incline 0.23%), a first climb (5.5 km, 4.19%), a downhill part (3.1 km, -6.79%), a second flat part (8.6 km, 0.25%) and the final climb (4.15 km, 3.13%). Volunteers' body weight was measured before each trial for software-based calculation of the appropriate resistance. After warming up for a minimum of 10 min volunteers could rest again and start the test whenever they felt ready. Throughout the test volunteers were free to manually change gears, drink and eat ad libitum, and were allowed visual feedback on the computer screen that displayed their speed and covered distance. The rear wheel of each bike was fitted with a power meter (Powertap Highly trained+, CycleOps, Madison, WI), which monitored power output over the whole course. Volunteers were vigorously encouraged during all tests.

Exercise efficiency. We assessed $\dot{V}O_2$ and respiratory exchange ratio (RER) at an absolute set submaximal steady-state cycling. Breath-by-breath values during the last minute of 5-min increments (150 W and 200 W for men; 100 W and 150 W for the woman) were averaged. Energy expenditure (EE) per minute was calculated from submaximal $\dot{V}O_2$ values and RERs (57). Delta efficiency (DE) was then calculated as the ratio of the change in work (kcal/min) by the change in energy expended (kcal/min) at the two submaxi-

mal workloads multiplied by 100: $DE (\%) = (\Delta \text{Work rate} / \Delta EE) \cdot 100$.

Arterial blood measurements. After local anesthesia with 2% lidocaine, a 20-gauge catheter (model 80115.09R, Vygon laboratories, Ecouen, France) was inserted percutaneously using the Seldinger technique into the radial artery. Arterial blood was sampled anaerobically in heparinized syringes and immediately analyzed for hemoglobin concentration ([Hb]), O₂ saturation (SaO₂), O₂ tension (PaO₂), and lactate concentration by means of the ABL 800 Flex (Radiometer, Copenhagen, Denmark). Blood O₂ content (CaO₂) was computed from the following formula: $CaO_2 = (1.34 \times [Hb] \times SaO_2) + (0.003 \times PaO_2)$. Arterial samples were collected at rest, 150 W, 200 W, and at exhaustion.

Intravascular volumes. Hemoglobin mass (Hb_{mass}) was quantified by a modified (56) carbon monoxide (CO)-rebreathing technique (11). After the subject had stayed for 20 min in a semirecumbent position, 2 ml of blood was sampled from an antecubital vein without stasis through a 20-gauge catheter and immediately analyzed in quadruplicate for 1) percent carboxyhemoglobin (%HbCO) and hemoglobin concentration ([Hb]) on a hemoximeter (ABL 800 Flex, Radiometer, Copenhagen, Denmark), and 2) hematocrit (4 min at 13,500 rpm). A bolus (1.5 ml/kg) of 99.997% CO (CO N47, Air Liquide) containing <0.1 ppm of nickel tetracarbonyl or ferrous pentacarbonyl was then rebreathed for 8 min. At the end of the rebreathing period, another similarly obtained and analyzed 2-ml blood sample was obtained. The change in %HbCO between the first and second measurement was used to calculate Hb_{mass}, taking into account the amount of CO remaining in the rebreathing circuit at the end of the procedure (2.2%) (11). The RCV, blood and plasma volumes were derived from Hb_{mass}, [Hb], and hematocrit (11) as assessed by the same operator for the entire study. The Hb_{mass} values expressed the average of duplicate measurement. The coefficient of variation for Hb_{mass}, assessed from duplicate baseline during the lead-in period, and expressed as the percent typical error (i.e., SD of difference scores/ $\sqrt{2}$), was 2.6%.

Near-infrared spectroscopy (NIRS). Thigh (sO_{2LEG}) and cerebral (sO_{2BRAIN}) measures of tissue oxygenation were obtained by NIRS (NIRO-200, Hamamatsu, Japan). The method has been previously described (69). Briefly, both measurements assume a homogeneous medium and use spatially resolved spectroscopy coupled to an analytical solution of the diffusion equation (69). NIRS reflects primarily capillary oxygenation although the signal is also affected by the arterial and venous blood and, for the muscle, myoglobin. The NIRO-200 uses an internal calibration derived independently from the pathway length of the infrared light. Changes in cerebral sO_{2BRAIN} oxygenation were relative to the values obtained at rest and reported as cerebral ΔsO_{2BRAIN} . NIRS measurements in skeletal muscle provide the oxygenation status of the hemoglobin in the microvasculature of the tissue (with a small contribution from myoglobin in skeletal muscle) thereby reflecting the balance between oxygen consumption (drive to reduce oxygenation) and delivery (drive to maintain oxygenation). NIRS values have been shown to correlate well with femoral venous oxygen saturation and can serve as a substitute measure of oxygen extraction (21).

Middle cerebral artery velocity. Transcranial doppler (TCD) measurements of cerebral blood velocity were performed (Doppler-box, DWL, Überlingen, Germany). The TCD-transducer was fixed above the temporal bone with a headband to carry out measurements on the proximal part of the middle cerebral artery (MCA). To achieve the best signal-to-noise ratio both probe position and insonation depth (40–60 mm) were carefully adjusted for each individual. The MCA velocity was computed from the integral of the maximum frequency Doppler shifts over one heartbeat and averaged over 30 s.

Blood pressure. Arterial pressure was measured with a transducer (MLT844, AD Instruments, Australia) connected to the arterial line placed at the level of the heart (4th intercostal space). Blood pressure data were collected and stored with Powerlab (AD Instruments, Australia).

Muscle function. Volunteers were seated on a wooden plate with no backrest and the angle of the knee joint was between 85 and 95 degrees. A mark was made on the subject's thigh in relation to the edge of the chair to ensure precise repositioning. A strap was placed around the lower leg, just above the ankle joint. The strap was secured to a rigid pole that was attached to a strain gauge. The strain gauge was calibrated twice a day, once in the morning and again in the afternoon. Force recordings were sampled at 1 kHz. Self adhesive electrodes (5 × 9 cm, Platinum Neurostimulation Electrodes, Axelgaard, Fallbrook, CA) were placed on the rectus femoris muscle at 25% of the distance from spina iliaca anterior superior to the proximal part of patella. The electrodes were fixed by tape (Fixomull stretch, BSN medical GmbH, Hamburg, Germany) and remained in position for the rest of the experiment. This was done to ensure no measurement errors due to repositioning of electrodes and to reduce the time elapsed from the end of exercise to investigation. Five to ten sub-maximal and close to maximal contractions were performed prior to determination of the optimal stimulation intensity. The stimulation intensity used was 440 ± 70 mA with a range between 220 and 500 mA. The stimulations were delivered using a single 200- μ s pulse, and voltage was 400 V (Digitimer DS7AH, Digitimer, Hertfordshire, UK). Optimal stimulation intensity was determined by increasing stimulation current from 50 mA until the current was at least 20% higher than the current eliciting a maximal twitch response. At first, an optimal stimulation intensity was determined by rapid increments of stimulation currents. When an optimal intensity was determined a stimulation current vs. twitch force curve was constructed with increments of 50 mA or less to ensure that a maximal twitch response was obtained. After determination of the optimal stimulation intensity, the subject was asked to perform a 5-s maximal voluntary contraction (MVC) with stimulation occurring 3 s into the 5 s contraction (superimposed twitch, T_{WS}). Five seconds after the MVC another stimulation was performed to determine the twitch force (T_W). This procedure was repeated every minute, three times in total. Maximal values for MVC, T_{WS} , and T_W were recorded. The superimposed twitch force was related to the twitch force recorded 5 s after the MVC and termed percent voluntary activation: $VA\% = [1 - (T_{WS}/T_W)] \times 100$. One minute after the third MVC the subject was asked to generate ~50% of the force recorded during MVC. When the force was constant after 5–20 s a stimulation was performed. After another minute of rest, the procedure was repeated at ~75% of MVC. The measurements were performed to ensure that the applied procedure could be used to assess %VA. The control measurements revealed %VA to be 64.3 ± 12.3 and 84.2 ± 9.1 at 50% and 75% of MVC, respectively. After the time trial, volunteers were rapidly moved to the wooden plate and repositioned as they were previously. After 45 s, a 5-s MVC was performed with a stimulation again being delivered 3 s into and 5 s after the MVC, as described above. The procedure was repeated three times starting every 30 s. Following 5 min of recovery the same series of 3 MVC and stimulations were performed. This protocol was used to assess central (neuromuscular) vs. peripheral (skeletal muscle) fatigue following time trial performance. The aim prior to exercise was to determine maximal twitch force. Accordingly, sufficient time, 60 s, was allowed to elapse between stimulations to avoid stimulation-induced fatigue. Following exercise, however, the aim was to examine whether a loss of twitch force had occurred with concern that maximal twitch force could recover during the elapsed time between measurements. Therefore the compromise was to allow only 30 s between stimulations after exercise. Since we used the maximal obtained twitch force in the calculations there is no error in the interpretation of the data with this approach.

Skeletal muscle biopsy. Under local anesthetics using the Bergström technique (6) with a needle modified for suction, skeletal muscle biopsies were obtained while the subject was at rest and a minimum of 24 h following last exercise training bout. The biopsy was dissected free of fat and connective tissue and divided into sections for mitochondrial respiration and muscle buffer capacity measurements. The section used for muscle buffer capacity was frozen immediately.

Mitochondrial respiration. A subsample of the biopsy (~20 mg) was sectioned into four parts to measure mitochondrial respiration. Each part was immediately placed in ice-cold biopsy preservation solution (BIOPS) containing 2.77 mM CaK_2EGTA buffer, 7.23 mM K_2EGTA buffer, 0.1 μM free calcium, 20 mM imidazole, 20 mM taurine, 50 mM 2-(*N*-morpholino)ethanesulfonic acid hydrate (MES), 0.5 mM dithiothreitol, 6.56 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 5.77 mM ATP, and 15 mM phosphocreatine (pH 7.1). Muscle samples were then gently dissected using forceps and fibers were chemically permeabilized via incubation in 2 ml of BIOPS containing saponin (50 $\mu\text{g}/\text{ml}$) for 30 min (49). Before each sample was added to its respective respiration chamber, the wet weight was measured (XS205 DualRange Analytical Balance, Mettler-Toledo AG, Switzerland). Respiration measurements were performed in mitochondrial respiration medium 06 (MIR06) containing 0.5 mM EGTA, 3 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 60 mM K-lactobionate, 20 mM taurine, 10 mM KH_2P_4 , 20 mM HEPES, 110 mM sucrose, 1 g/l bovine serum albumin, and catalase 280 IU/ml (pH 7.1). Measurements of oxygen consumption were performed in duplicate at 37°C using the high-resolution Oxygraph-2k (Oroboros, Innsbruck, Austria) with all additions of substrates, uncouplers, and inhibitors added in series. All experiments were carried out in a hyperoxygenated environment to prevent any potential oxygen diffusion limitation. Two substrates, uncoupler, and inhibitor titration (SUIT) protocols were used in the study. Each protocol was specific to the examination of individual aspects of respiratory control through a sequence of coupling and substrate states induced via separate titrations. A more complete listing of and thorough explanations for the standard nomenclature regarding various respiratory states, SUIT protocols, coupling control, and flux control ratios can be found in detail elsewhere (67, 68). Briefly, oxidative phosphorylation (OXPHOS) capacity (P; usually compared with originally defined state 3 respiration) is the ADP-activated mitochondrial respiratory state of oxidative phosphorylation with saturating concentrations of ADP, inorganic phosphate, oxygen, and defined reduced substrates. Mitochondrial electron transfer system capacity, E, is the experimentally induced noncoupled (fully uncoupled) state, in which ADP, inorganic phosphate, oxygen, and defined substrates are present at saturating levels along with the titration of an established amount of uncoupler to optimum concentrations facilitating maximal noncoupled respiration through the electron transport system (ETS) (67).

The specific SUIT protocol was used to measure OXPHOS capacity (P) in the permeabilized skeletal muscle preparations. Maximal values of P were induced with the additions of malate (2 mM), octanoyl carnitine (0.2 mM), glutamate (10 mM), succinate (10 mM), and saturating adenosine diphosphate concentrations (ADP; 5 mM). Such a protocol provides saturating concentrations of substrates for both NADH dehydrogenase, complex I, and succinate dehydrogenase, complex II. The integrity of the outer mitochondrial membrane was assessed with the addition of cytochrome *c* (10 μM). The addition of cytochrome *c* had no additive effect on respiration with negligible increases of 3.4%, respectively, confirming intact and viable mitochondria in the skeletal muscle samples. Maximal noncoupled ETS respiration (E) was then measured following the titration of carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP; a total of 1.5 μM in steps of 0.5 μM). Last, residual oxygen consumption, indicative of nonmitochondrial oxygen consumption, was assessed following

the inhibition of complexes I and III with the addition of rotenone (0.5 μM) and antimycin A (2.5 μM), respectively.

Citrate synthase activity. Citrate synthase (CS) activity was assayed in homogenates of all skeletal muscle samples used for respiration measurements. The content of the oxygraph chamber (2 ml) was removed after each respiration experiment and washed twice with 2 ml of MiR06. The solutions were combined, homogenized for 60 s with an Ultra-Turrax homogenizer at maximum speed, frozen in liquid nitrogen, and stored at -80°C . The activity of citrate synthase was measured spectrophotometrically at 412 nm and 37°C . Eight-hundred microliters of homogenate was added to 200 μl medium containing 0.1 mM 5,5-dithio-bis-(2-nitrobenzoic) acid (DTNB), 0.5 mM oxaloacetate, 0.31 mM acetyl coenzyme A, 5 mM triethanolamine hydrochloride, 50 μM EDTA, and 0.1 M Tris-HCl (pH 8.1) (81).

Skeletal muscle buffer capacity. The method was previously described in Ref. 39. After having adjusted pH of the sample to 7.1 with 0.01 M NaOH, the sample was titrated to pH 6.0 by serial additions of 0.01 M HCl followed by titration back to pH 7.1 by serial additions of 0.01 M NaOH. The pH was measured after each addition. The non- HCO_3^- physicochemical buffer capacity was determined from the number of moles of H^+ required to change pH from 7.1 to 6.5 and was expressed as micromoles H^+ per kilogram dry weight per unit of pH (59). The in vivo muscle buffer capacity was calculated as the change in muscle lactate from rest to exhaustion divided by the change in muscle pH (79).

Statistics

From the studies we obtained 6 intravascular volumes, 22 measures of mitochondrial function, 40 measures of pulmonary gas exchange, 6 measurements of exercise efficiency, 24 arterial blood gas or metabolite measurements, 36 measures of cardiac performance and blood pressure, 4 measurements of cerebral blood flow, and 14 measures of vastus

lateralis and cerebral oxygenation. In total 152 different measurements were recorded and all were the average of 2–6 separate exercise tests. We recorded two separate measures of exercise performance, and these are presented along with $\dot{V}\text{O}_{2\text{max}}$ for our 16 volunteers in Table 1.

The first objective of the analysis was to reduce the number of variables to <16 to have sufficient degrees of freedom to perform a statistical analysis. Of the 152 potential determinants we excluded 59 variables because they were either redundant (i.e., showed high correlation with a related variable) or because no a priori hypothesis could be justified. This left 73 variables for further evaluation for the main analysis. Those variables were grouped into six groups relating them to efficiency, pulmonary performance, cerebral factors, muscular factors, cardiac-vascular factors, or oxygen transport. If appropriate, each variable was allowed to enter two groups as long as the total number of variables in one group did not exceed 15 ($n - 1$). Following calculation of a nonparametric correlation matrix (Kendall's tau-b), we performed a multiple stepwise regression (Pearson) of the principal factors in each group to identify the variables for which the majority of the variance could be attributed. Factor analysis describes variability among observed variables in terms of a potentially lower number of unobserved variables called factors. The underlying unobserved, or latent, variable, however, is not of interest for this study and principal factor analysis was simply used to reduce the number of variables entering the analysis. Only the variables with loadings on the first three principal factors larger than 0.6 were considered for further analysis. The resulting variables selected are presented in Fig. 1.

RESULTS

Maximal Incremental Power Output

Following multiple stepwise regression analysis for determinants of maximal incremental power output, Hb_{mass} was identified as best single parameter ($r^2 = 0.48$, $P = 0.0027$).

Table 1. Measures of exercise performance along with $\dot{V}\text{O}_{2\text{max}}$

Subject	Height, cm	Weight, kg	$\dot{V}\text{O}_{2\text{max}}$, $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$		TT _{mean} , W/kg		Incremental _{max} , W/kg	
			Ranking	Value	Ranking	Value	Ranking	Value
<i>First quartile</i>								
<i>O</i>	177	70	<i>1</i>	80.44	7	4.35	2	6.31
<i>B</i>	180	72	2	77.32	8	4.34	7	5.99
<i>C</i>	176	65	3	76.87	1	5.06	1	6.89
<i>L</i>	193	76	4	74.30	2	4.62	3	6.28
<i>Second quartile</i>								
<i>I</i>	173	62	5	72.24	4	4.50	9	5.88
<i>F</i>	176	62	6	71.95	6	4.43	5	6.08
<i>P</i>	175	76	7	71.79	10	3.98	10	5.86
<i>S</i>	164	63	8	71.51	12	3.91	15	4.89
<i>Third quartile</i>								
<i>N</i>	178	65	9	71.44	5	4.48	4	6.20
<i>M</i>	173	60	10	69.39	14	3.64	8	5.94
<i>G</i>	188	74	11	69.20	3	4.52	6	6.05
<i>H</i>	183	69	12	68.36	9	4.14	11	5.82
<i>Fourth quartile</i>								
<i>Q</i>	183	80	13	68.00	16	3.60	14	5.37
<i>K</i>	170	59	14	64.15	13	3.74	12	5.79
<i>T</i>	182	76	15	63.12	11	3.93	13	5.55
<i>J</i>	194	87	16	58.33	15	3.61	16	4.88
Mean	179	70		70.53		4.18		5.86
SD	8	8		5.57		0.42		0.51

Numbers in italics preceding values identify subjects according to their ranking, e.g., subject *O* ranks 1st in maximal oxygen consumption ($\dot{V}\text{O}_{2\text{max}}$), 7th in average time trial power (TT_{mean}), and 2nd in maximal power output during an incremental test (Incremental_{max}).

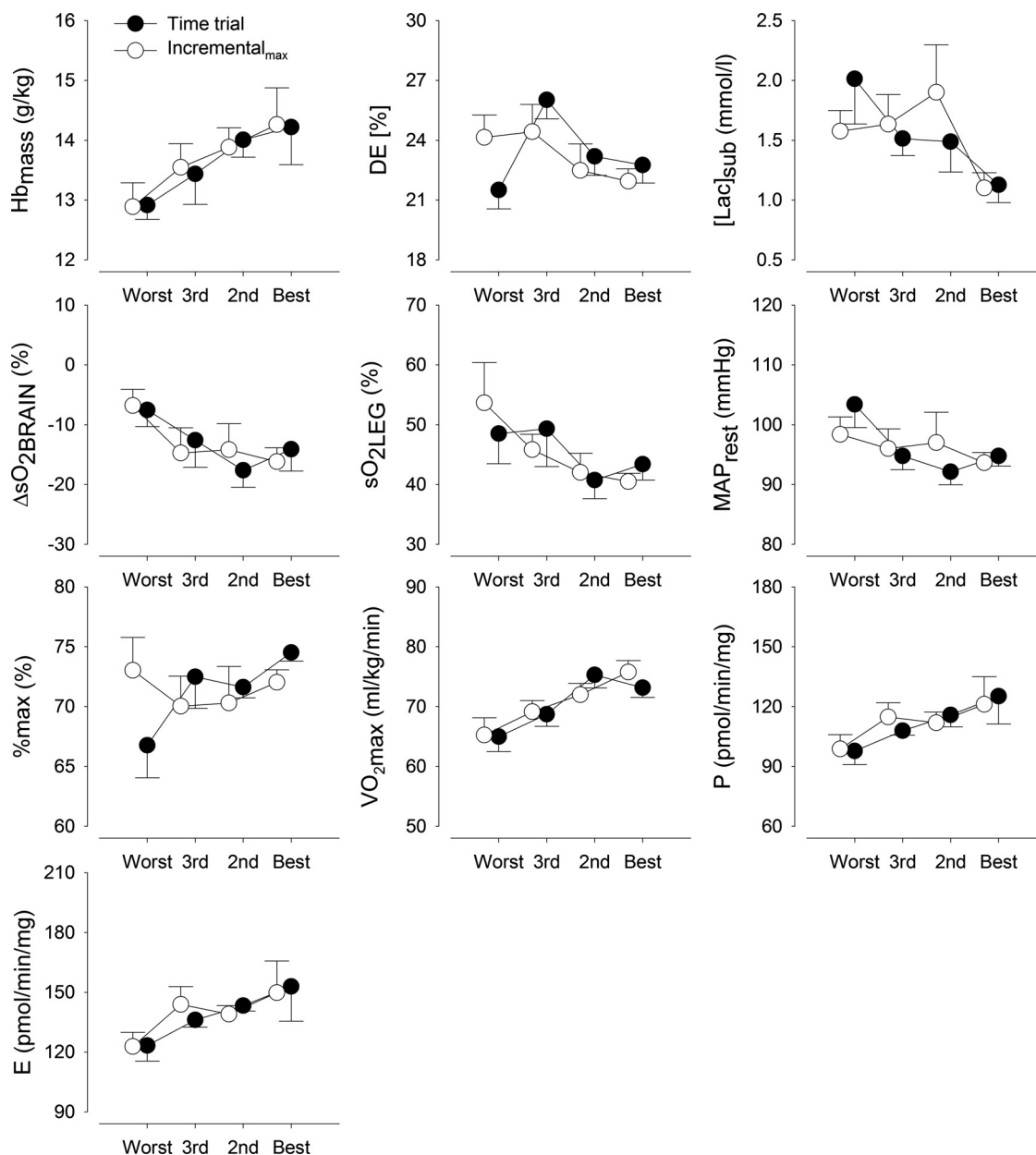


Fig. 1. Predictor variables correlation with exercise performance in highly trained cyclists. Ten principal factors of exercise performance (y-axis) plotted according to exercise performance ranked quartiles from worst to best (x-axis). Range of exercise performance (W/kg) in each quartile was 3.60–3.74, 3.91–4.14, 4.34–4.48, and 4.50–5.06 for worst to best average time trial power outputs (black circles); and 4.88–5.55, 5.79–5.88, 5.94–6.08, and 6.20–6.89 for worst to best maximal incremental power outputs (open circles). MAP_{rest}, resting mean arterial pressure; P, maximal oxidative phosphorylation capacity in skeletal muscle; E, electron transport system capacity in skeletal muscle; DE, percentage ratio of the change in work performed per minute (5 min at 150 W to 5 min at 200 W) to the change in energy expended per minute; sO₂, tissue oxygenation; ΔsO₂, changes in tissue oxygenation from rest to maximal exercise at the end of the incremental test; %max, workload during time trial as percent of maximal power output during the incremental test; Hb_{mass}, total body hemoglobin; [Lac]_{sub}, mean blood lactate concentrations during 5 min at 150 W and 5 min at 200 W; VO₂max, maximal oxygen consumption. Data are presented as means ± SE.

Best two-parameter model included $\dot{V}O_{2\max}$ ($r^2 = 0.69$, $P = 0.0005$). The best fit three-parameter model, 78% ($P = 0.0003$) of the variation in maximal incremental power output, could be explained by Hb_{mass} ($P = 0.0038$), $\dot{V}O_{2\max}$ ($P = 0.0213$), and sO₂LEG ($P = 0.0463$).

Time Trial Performance

We performed multiple stepwise regression analysis on the selected parameters and mean power output during the time trial.

This analysis revealed that for a single-parameter model the oxidative phosphorylation capacity of the skeletal muscle, P, provided the best fit ($r^2 = 0.47$, $P = 0.0035$). For a two-parameter model submaximal lactate concentration improved the fit significantly ($r^2 = 0.68$, $P = 0.0006$). Finally, for the best fit three-parameter model, 78% ($P = 0.0002$) of the variation in time trial performance could be explained by P ($P = 0.0005$), submaximal blood lactate concentrations ($P = 0.0017$), and the leg oxygenation (sO₂LEG) at exhaustion ($P = 0.0295$).

Overall Exercise Performance

Performance of our two functional tests of exercise capacity strongly correlated with one another. Average power output during the time trial correlated with maximal power output during the incremental exercise test ($r^2 = 0.82$, $P < 0.001$).

Finally, we performed cluster analyses on the correlation matrix between performance parameters and predictor variables. For this we included the 10 main predictors as well as 9 “second tier” variables last excluded by the principal factor analysis. We clustered variables on their correlation strength (Kendall’s tau) with measures of aerobic performance, i.e., time trial mean power output and maximal incremental power output. This analysis revealed that overall exercise performance positively correlates best (Fig. 1) with parameters of oxygen transport ($\dot{V}O_{2\max}$, Hb_{mass}), extraction ($sO_{2\text{LEG}}$), and skeletal muscle oxidative capacity (both P and electron transport system capacity, E).

DISCUSSION

This study presents several main findings regarding specific determinants of exercise performance: 1) Hb_{mass} correlates the strongest with maximal incremental power output; 2) maximal oxidative phosphorylation capacity of the skeletal muscle, P, is the strongest determining factor relating to 26-km time trial performance; and 3) primary factors determining overall (time trial and maximal incremental power output) include measures regarding the capability for oxygen transport, high $\dot{V}O_{2\max}$ and Hb_{mass} , in addition to measures of oxygen utilization, P and electron transport system capacities, E, in the skeletal muscle.

Maximal Incremental Power Output

Overall, 78% of the variance in maximal incremental power output was attributable to, in order of significance, Hb_{mass} , $\dot{V}O_{2\max}$, and $sO_{2\text{LEG}}$. An individual’s $\dot{V}O_{2\max}$ establishes the upper limit of aerobic capacity and is a requirement for highly trained endurance capability (72, 74) with oxygen consumption proportion to exercise workload. This study confirms previous findings in showing that $\dot{V}O_{2\max}$ is a significant correlate to maximal incremental power output. Convective limitations in oxygen delivery appear to be the primary physiological bottleneck of maximal oxygen consumption in humans (1, 4, 45). Maximal oxidative phosphorylation capacity, P, in the skeletal muscle has been shown to exceed the upper limit of oxygen delivery during whole body maximal exercise (8) and thus would not be expected as a significant correlate of maximal incremental whole body exercise. The results from the present study agree with and emphasize the importance of convective oxygen capacity for maximal exercise intensities. Hb_{mass} is the physiological factor that correlates the strongest to maximal incremental power output in highly trained endurance athletes accounting for 48% of the variation. This supports previous findings that have shown strong relationships between Hb_{mass} and $\dot{V}O_{2\max}$ (28, 47, 61). While total blood volume is also understood to complement Hb_{mass} (47, 61, 77) we did not examine the correlation between total blood volume and maximal incremental power output. Of the variables we excluded from the potential 152 variables of interest, blood volume was among them because of its redundancy (high correlation inherently resulting from method of calculation) with Hb_{mass} . Con-

vective transport of oxygen is also highly reliant on the pumping capacity of the heart as maximal cardiac output is understood to be a limiting factor at maximal workloads engaging a significant percentage of total body muscle mass (1, 52). As we did not directly assess cardiac output in the present study, high $\dot{V}O_{2\max}$ values and the capacity for oxygen delivery to the leg are both indirectly indicative of high cardiac capacity and support maximal cardiac output as a dominant determinant in maximal aerobic power output. Cardiac output and leg blood flow both increase in parallel with an increase in work output (12, 76). Accordingly, although not directly measured, the significance of maximal cardiac output capacity is indirectly suggested as vital to maximal incremental power output in the present study.

The third strongest correlate to maximal incremental power output in the best fit three-parameter model was $sO_{2\text{LEG}}$. Muscle oxygen extraction increases with increasing workload during exercise (12). Maximal values of oxygen extraction averaging 91% have been observed in healthy cyclists during maximal exercise with values ranging from 87% to as high as 95% (27). As oxygen extraction is known to improve with training (73) the present study demonstrates its relation to maximal power output in highly trained athletes.

Time Trial Performance

The present study demonstrates that skeletal muscle oxidative capacity is the strongest correlate to exercise endurance among highly trained cyclists (average $\dot{V}O_{2\max} = 70.53 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; Table 1), accounting for 47% of the variation in 26-km time trial performance. This specific mitochondrial state, P, demonstrates the mitochondrial capacity to catalyze a sequential set of redox reactions that are partially coupled to the production of ATP via ATP synthase. Mitochondrial enzymes, content, volume density, and respiratory capacity all increase linearly in response to training (19, 23, 34–36, 83) and are elevated in trained vs. untrained counterparts (5, 23, 26, 37, 50, 51) as well as in moderate vs. highly trained athletes (17). Acute reduction of mitochondrial oxidative capacity limits maximal oxygen consumption in skeletal muscle (31, 62, 71). Exercise training has a direct dose response on endurance performance, skeletal muscle respiratory capacity, and mitochondrial markers in skeletal muscle of rats (23); however, these animals varied significantly in level of training, unlike the athletes in the present study. Homogeneous groups of athletes have displayed differences in exercise efficiency (75), lactate threshold, and endurance performance (16) without differences in static measurements of mitochondrial content. When normalizing mitochondrial respiratory rates to skeletal muscle CS concentration, respiratory differences between trained and untrained groups are ostensibly lost (23, 63). The present study differentiates the importance of dynamic mitochondrial properties (e.g., maximal oxidative phosphorylation and electron transport system capacities) on exercise performance as opposed to previously quantified static mitochondrial characteristics such as CS activity, which is used as a common measure of mitochondrial content. CS activity did not significantly correlate with time trial performance in the present study, highlighting the importance of functional mitochondrial analyses in combination with instantaneous “snap-shot” measurements of individual mitochondrial characteristics.

Submaximal arterial lactate concentrations, along with oxidative phosphorylation capacity, accounted for 68% of the variation in time trial performance. The ability to maintain a given workload with lower blood lactate concentrations correlates with endurance performance in trained individuals (17, 22, 53, 55, 70, 75) and this study confirms these results. World class cyclists have been reported to maintain blood lactate concentrations below 3 mM while cycling for 20 min at 80% of their maximal workload: $\sim 86\%$ of $\dot{V}O_{2\max}$ (54). Despite similar aerobic capacities, cyclists with higher lactate thresholds were able to maintain exercise at 88% of their $\dot{V}O_{2\max}$, which happened to be at a slightly higher absolute power output, for twice as long as those with comparatively low lactate thresholds (16). The single strongest predictor of performance has been repeatedly attributed to $\dot{V}O_{2\max}$ percentage at lactate threshold (16, 55).

Trained individuals oxidize more fat and less carbohydrate than untrained counterparts at a given submaximal workload (10, 32). As the subjects who performed best on the 26-km time trial exercise test in the present study typically presented with, respectively, lower arterial lactate concentrations for a given submaximal workload (Fig. 1), those values did not, however, correlate with quantification of fat oxidation via respiratory exchange ratio values ($R^2 = 0.02$; data not presented). Furthermore, maximal octanoyl-carnitine stimulated respiration capacity, representative of the capacity for fatty acid oxidation and maximal electron flow through electron-transferring-flavoprotein dehydrogenase in the inner mitochondrial membrane, neither differed between subjects nor did values correlate with exercise performance. We included all relevant respiratory states in the factor analysis (1st step) and only used those that could contribute for a large part of the variation in the data set for the stepwise regression analysis (2nd step). Thus we did examine measures of maximal fat oxidative capacity in the analysis and they were not "significant" in relation to exercise performance. Thus the oxidative potential of the skeletal muscle can be thought to directly relate to blood lactate measurements more so than nutrient partitioning. More robust oxidative capacities in skeletal muscle limit the disproportionate increase in glycolytic flux at greater workloads. This, in turn, maintains a lower lactate concentration, as lactate production is more effectively matched by pathways of lactate disposal (84), explaining why skeletal muscle oxidative capacity and low arterial lactate concentrations, together, correlate well with time trial performance.

Human skeletal muscle respiratory capacity, more so than fiber type, corresponds with time to fatigue during short intensive exercise (41). Although fiber type is believed to be important to endurance performance as exercise efficiency has been primarily attributed to both percentage of type I fibers (18, 29, 38, 48, 63) along with concentration of uncoupling protein isotype 3 (63), it is the capillary density of the muscle that is directly proportional to the respiratory capacity of that muscle and not the fiber type (40). The importance of exercise efficiency and its role on exercise performance has oft been a topic of discussion (7, 15, 18, 38, 42, 43, 54, 64, 65). Suggested increases in efficiency of 1% has been proposed and to drop ~ 63 s off a 40-km time trial performance (43) while an 1.8% greater efficiency has shown to parallel a 9% improvement in work output obtained during a 1-h cycling performance test (38). However, most empirical evidence demonstrates no dif-

ference in efficiency between elite, trained, or novice athletes (7, 60, 64, 66, 82), denoting that exercise efficiency is not a predictor of endurance performance in elite level cycling (64). The present study calculated DE via two relatively low-intensity power outputs (150 and 200 W, corresponding to 50% and 63% $\dot{V}O_{2\max}$, respectively). This may have introduced more variability in DE values compared with those if more measurements during steady-state exercise ranging from 50 to 90% $\dot{V}O_{2\max}$ were collected. Regardless, this study confirms and supports previous findings as DE was not identified as a primary contributing physiological factor of endurance performance among a homogeneous group of highly trained athletes.

The third strongest correlate with time trial performance was $sO_{2\text{LEG}}$ as the stronger athletes were able to reduce tissue oxygenation the greatest (Fig. 1). Oxygen extraction significantly correlates with oxygen consumption over various workloads (13) and oxidative capacity in the skeletal muscle has been proposed to assist in facilitating the extraction of oxygen during exercise (13). Measurement of $sO_{2\text{LEG}}$ was the only variable strongly predictive of both maximal incremental power output and time trial performance. The relationship between oxygen extraction and oxidative capacity of the muscle is not well understood and requires further investigation.

Overall Exercise Performance

This study demonstrated a high correlation of performance between exercise tests in highly trained athletes. Maximal incremental power output and time trial performance are strongly correlated to one another ($r^2 = 0.82$, $P < 0.001$). Peak power output obtained in these subjects during an all-out 30-s sprint was also significantly correlated to maximal incremental power outputs and time trial performance ($P < 0.01$, data not shown). Peak power output has been reported to correlate highly with time trial performance (30) but seemingly more so with average power output than time to completion of a time trial (3). Although percent of maximal power output averaged during time trial was 1 of the 10 best correlates of exercise performance in the present study (Fig. 1), other variables were observed to have stronger validity as determinants of exercise performance.

The physiological variables most predictive of overall exercise performance included parameters relating to both the transport of oxygen and the capacity for cellular oxygen utilization (Fig. 1). These data support other studies identifying the collective importance of oxygen delivery and oxidative capacity in regulating oxygen flux (31). The cluster analysis supports regression analyses, as both P and E were strongly predictive of overall exercise performance in highly trained athletes along with $\dot{V}O_{2\max}$ and Hb_{mass} . In the specific mitochondrial state E, inner mitochondrial membrane potential is collapsed with an open transmembrane proton circuit and thus serves as an indication of mitochondrial membrane potential (25, 67). As the transport of oxygen to the working skeletal muscle during exercise is firmly recognized as an essential factor determining performance, this study develops our understanding of the limitations to exercise performance in highly trained athletes by illustrating the importance coupling a high oxygen transport capacity with an equally sizable ability for cellular oxygen utilization.

Conclusion

In summary, this study identified several individual and specific physiological variables detailing the determinants of exercise capacity in highly trained cyclists. The primary predictors for time trial performance and maximal incremental power output are skeletal muscle oxidative phosphorylation capacity, P, and Hb_{mass}, respectively. Overall exercise performance is strongly attributed to means of oxygen transport (Hb_{mass}), ability to extract oxygen in the skeletal muscle (sO_{2LEG}), and the capacity for oxygen utilization within the muscle (P, E).

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DISCLOSURES

E. Gnaiger is the founder and managing director of Orobos Instruments, high-resolution respirometry, Scharpfstrasse 18, A-6020 Innsbruck, Austria.

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