Muscle Oxygen Supply and Use in Type 1 Diabetes, From Ambient Air to the Mitochondrial Respiratory Chain: Is There a Limiting Step?

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OBJECTIVE

Long before clinical complications of type 1 diabetes (T1D) develop, oxygen supply and use can be altered during activities of daily life. We examined in patients with uncomplicated T1D all steps of the oxygen pathway, from the lungs to the mitochondria, using an integrative ex vivo (muscle biopsies) and in vivo (during exercise) approach.

RESEARCH DESIGN AND METHODS

We compared 16 adults with T1D with 16 strictly matched healthy control subjects. We assessed lung diffusion capacity for carbon monoxide and nitric oxide, exercise-induced changes in arterial O_2 content (SaO_2, PaO_2, hemoglobin), muscle blood volume, and O_2 extraction (via near-infrared spectroscopy). We analyzed blood samples for metabolic and hormonal vasoactive moieties and factors that are able to shift the O_2-hemoglobin dissociation curve. Mitochondrial oxidative capacities were assessed in permeabilized vastus lateralis muscle fibers.

RESULTS

Lung diffusion capacity and arterial O_2 transport were normal in patients with T1D. However, those patients displayed blunted exercise-induced increases in muscle blood volume, despite higher serum insulin, and in O_2 extraction, despite higher erythrocyte 2,3-diphosphoglycerate. Although complex I– and complex II–supported mitochondrial respirations were unaltered, complex IV capacity (relative to complex I capacity) was impaired in patients with T1D, and this was even more apparent in those with long-standing diabetes and high HbA_{1c}. $\dot{V}O_{2max}$ was lower in patients with T1D than in the control subjects.

CONCLUSIONS

Early defects in microvascular delivery of blood to skeletal muscle and in complex IV capacity in the mitochondrial respiratory chain may negatively impact aerobic fitness. These findings are clinically relevant considering the main role of skeletal muscle oxidation in whole-body glucose disposal.

Large clinical trials related to type 1 diabetes have underlined the important role of the prolonged exposure of tissues to hyperglycemia in the pathogenesis of microvascular complications (1). Endothelial dysfunction can occur very early in the disease, that is, before overt vascular complications occur (2), hence altering metabolites and oxygen (O_2) supplied to major tissues. Hyperglycemia may also

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contribute to mitochondrial dysfunction, leading to impaired energy production in tissue (3).

Long before overt clinical complications of diabetes develop, oxygen supply and use can be challenged in daily-life situations such as aerobic exercise. $\dot{V}O_{2max}$, determined during exhaustive incremental exercise, reflects the highest achievable outcome of the integrated pathway. It relies on the serial steps of oxygen transfer from the lungs to blood, the delivery of oxygenated blood through complicated branching networks of blood vessels, and the final use of oxygen in mitochondria in skeletal muscle. Several studies of type 1 diabetes have attempted to investigate some of these serial steps, albeit each with an isolated approach.

Although a low pulmonary diffusion capacity has been described in patients experiencing long-term diabetes complications (4), this does not appear as clearly in studies including uncomplicated patients (5,6), possibly because of the wide range of glycemic control among patients (7). The lungs represent a suitable target for hyperglycemia-induced vessel dysfunction and nonenzymatic glycation of collagen proteins because of their wide capillary network and the significant amount of connective tissue they contain. Concerning the second step of the oxygen supply process, we previously suggested normal arterial oxygenation but impaired exercise-induced muscle vasodilatation in uncomplicated patients with poorly controlled type 1 diabetes (8).

The ultimate step of O_2 utilization in the mitochondria has been only partially investigated in humans with type 1 diabetes. Noninvasive in vivo approaches using either near-infrared spectroscopy (NIRS) (i.e., muscle oxygen extraction) during aerobic exercise (8) or ^{31}P-MRS (i.e., calculating the maximal rate of ATP oxidative resynthesis) following a local isometric exercise (9–11) allowed the possibility to identify impaired muscle extraction, impaired mitochondrial use of oxygen, or both in patients with type 1 diabetes, and more so in those with a high HbA_{1c} level. However, the exact causes for lower in vivo muscle oxygen extraction, use, or both cannot be inferred from these indirect, noninvasive approaches. Studies using muscle biopsies reported normal maximal oxidative enzymatic capacities in subjects with type 1 diabetes (10,12–14). Nevertheless, enzymatic assays of the individual steps of the Krebs cycle, β-oxidation, and respiratory chain complexes cannot reveal how well all enzymes interact with each other and may mask some mitochondrial defects. In contrast, in permeabilized muscle fibers, in situ gold-standard experiments using a specific substrate/inhibitor titration approach provide detailed characterizations of functional, intact mitochondria in their normal intracellular position and assembly, preserving essential interactions (15). Monaco et al. (16) implemented this method to clarify mitochondrial (dys)function in type 1 diabetes and found decreased complex II–supported respiration. However, they did not test in vivo the putative consequences of this ex vivo defect .

Therefore, by combining multiple in vivo (particularly during exercise) and ex vivo (in muscle biopsies) approaches in patients and their strictly matched healthy control subjects, this study aims to gain further in-depth insight into the impact of type 1 diabetes and glycemic control on all steps of the integrated pathway for oxygen, from the atmosphere to the mitochondrial respiratory chain in skeletal muscle.

RESEARCH DESIGN AND METHODS

This study was approved by the North Western IV regional ethics committee (no. EudraCT: 2009-A00746–51). Written consent was obtained from patients before their inclusion in the study. Sixteen patients (18–40 years of age) who had type 1 diabetes for at least 1 year and were free from microvascular and macrovascular complications were recruited (T1D group) (Table 1). They were compared with 16 healthy subjects with normal glucose tolerance, as checked with an oral glucose tolerance test OGTT and based on World Health Organization criteria (control [CON] group). The healthy subjects were selected (through verbal questioning) to strictly match each of the patients in the T1D group according to sex and to preestablished ranges or values for age (±7 years), BMI (±4 kg·m^{-2}), moderate to vigorous leisure time physical activity level (±1 h·week^{-1} when the patient's physical activity category was 0 h·week^{-1}, ±2 h·week^{-1} for the category of 2–6 h·week^{-1}, and ±4 h·week^{-1} for the category of >6 h·week^{-1}; patient-control pairs were in the same category), and tobacco use (no smoking, <10 cigarettes a day, and >10 cigarettes a day). The matching of participants in terms of body composition and physical activity was checked further by using DEXA (Hologic, Inc.), the validated Modified Activity Questionnaire (17), and accelerometry (GT1M activity monitor; ActiGraph) over seven consecutive days.

Subjects came twice to the laboratory. We requested that they refrain from vigorous activity for 48 h before each visit and from using tobacco the morning of each visit.

During the first visit, patients with type 1 diabetes received their usual morning insulin bolus, and all subjects consumed a breakfast (based on their usual breakfast but containing a mean ± SD of 8.1 ± 4.7% protein, 43.3 ± 16.1% lipid, 48.6 ± 15.2% carbohydrate) that had been verified by a dietitian. Afterward, we assessed lung diffusion capacity for carbon monoxide (DL_{CO}) and nitric oxide (DL_{NO}). An incremental maximal cycling exercise was performed 3.4 ± 0.5 h after breakfast, and respiratory gas exchange, arterial O_2 transport, skeletal muscle perfusion, and O_2 extraction were measured concomitantly. After the patient rested for 2 min while sitting on the cycle ergometer (Excalibur Sport; Lode, Groningen, the Netherlands) (baseline), the test started at 30 W and was increased by 20 W every 2 min until exhaustion; it was performed at an ambient temperature (18−20°C).

On the morning of the second visit, after an 8-h overnight fast, a muscle biopsy was taken from the vastus lateralis to assess ex vivo intrinsic mitochondrial respiratory capacity in permeabilized skinned muscle fibers.

Alveolar-Capillary Membrane Diffusion Capacity

DL_{CO} was assessed following international guidelines; apnea was maintained for at least 8 s (gas sensor device, Medisoft, Dinant, Belgium). In order to access the determinants of DL_{CO} (i.e., membrane transfer capacity [D_m] and capillary lung volume [$\dot{V}_c$]), DL_{NO} was evaluated further.

Table 1—Participant characteristics

	T1D (n = 16)	CON (n = 16)
Anthropometric and demographic data		
Sex		
Male	12	12
Female	4	4
Age (years)	28.5 ± 6.8	27.7 ± 6.6
BMI (kg · m^{-2})	22.9 ± 2.2	23.1 ± 2.3
Smoking status		
Smoker	4	4
Nonsmoker	12	12
Fat mass (%)	19.8 ± 6.4	18.4 ± 5.6
Fat mass of right leg (%)	20.7 ± 6.9	20.5 ± 7.8
HbA_{1c} (%)	8.3 ± 1.5**	5.2 ± 0.2
HbA_{1c} (mmol/mol)	67.0 ± 16.4**	33.0 ± 2.2
Diabetes duration (years)	8.5 ± 5.2	NA
Insulin delivery (MDI/CSII)		
MDI	10	NA
CSII	6	NA
Insulin dose (units·kg^{-1}·day^{-1})	0.67 ± 0.18	NA
Physical activity†		
Leisure activity per MAQ (h·week^{-1})	2.8 ± 3.3	3.0 ± 2.0
Leisure activity per MAQ (MET-h·week^{-1})	17.6 ± 16.7	21.6 ± 15.5
Total activity per MAQ (MET-h·week^{-1})	49.7 ± 93.7	45.0 ± 50.3
MVPA per accelerometry (min·week^{-1})	232.2 ± 204.5	264.5 ± 128.5
Sedentary time per accelerometry (h·day^{-1})	8.7 ± 2.2	10.9 ± 2.7
Usual daily macronutrient intake		
Total caloric intake (kcal·day^{-1})	1,992.6 ± 496.8	2,291.2 ± 489.5
Protein (% of total calories)	16.1 ± 3.1	16.3 ± 3.2
Fat (% of total calories)	33.5 ± 7.0	36.3 ± 3.7
Polyunsaturated fatty acid–to–saturated fatty acid ratio	0.3 ± 0.4	0.3 ± 0.4
Cholesterol (mg·day^{-1})	292.7 ± 146.1	335.3 ± 145.2
Carbohydrate (% of total calories)	50.4 ± 7.4	47.4 ± 5.2
High glycemic index carbohydrate (% of total calories)	14.8 ± 5.3	17.1 ± 4.3
Fiber intake (g·day^{-1})	18.9 ± 4.8	19.0 ± 4.8

Data are mean ± SD or number of patients. Patients were free from microvascular (retinopathy, nephropathy, neuropathy) and macrovascular (high blood pressure, coronary disease, peripheral arteriopathy) complications. Fat mass was measured by DEXA. We also checked that the subcutaneous skinfold was <1.5 cm at the vastus lateralis to ensure the accuracy of NIRS measurements. Just before exercise, we measured HbA_{1c} in EDTA anticoagulated blood (VARIANT II TURBO System; Bio-Rad); patients' HbA_{1c} levels ranged between 5.8% (40 mmol/mol) and 10.7% (93 mmol/mol). Accelerometry data (collected by a GT1M activity monitor; ActiGraph) are displayed only for eight subjects per group because one healthy control subject and four patients with T1D did not strictly follow our recommendations (mainly, wearing the accelerometer during all waking hours), and the accelerometer devices worn by two patients with T1D were defective (no signal was recorded at the end of the week). We assessed usual daily macronutrient intake using a 3-day diary (including two weekdays and one weekend day), which was checked by a research-trained dietitian during an appointment with the participant. CSII, continuous subcutaneous insulin infusion; MAQ, Modifiable Activity Questionnaire; MDI, multiple daily insulin injections; MVPA, moderate to vigorous physical activity; NA, not applicable. Values are significantly different from those of the CON group, per the Wilcoxon test, at $^{**}P < 0.01$. †P values (Wilcoxon test) for leisure activity (h·week^{-1} and MET-h·week^{-1}), total activity (MET-h·week^{-1}), and MVPA (min·week^{-1}, per accelerometry) were 0.53, 0.43, 0.64, and 0.26, respectively.

Cardiopulmonary Response

Electrocardiography was performed and pulmonary gas exchanges were measured continuously throughout exercise using an Ergocard breath-by-breath system. $\dot{V}O_{2max}$(the highest 15-s mean value upon termination of the test) was obtained for all subjects (Table 2). O_2 pulse (the ratio of $\dot{V}O_2$ to heart rate) throughout exercise was used as an indicator of stroke volume (18).

Muscle Perfusion and O_2 Extraction

Subjects were equipped with an NIRS probe (Oxymon MkIII; Artinis Medical Systems, Gelderland, the Netherlands) to monitor, at 10 Hz, light absorption (two continuous wavelengths: 780 and 850 nm) across the vastus lateralis microvessels throughout exercise (8). Using the Beer-Lambert law and normalization to values from the baseline period, we determined changes in muscle oxygenation (oxyhemoglobin [ΔO_2Hb]), deoxygenation due to O_2 extraction (deoxyhemoglobin [ΔHHb]), and blood volume (total hemoglobin [ΔTHb], the arithmetical sum of ΔO_2Hb and ΔHHb) (19).

Blood Analyses

Venous blood samples were taken through a catheter in the forearm while the subjects were at rest and during maximal exercise in order to measure plasma glucose, catecholamines, serum free insulin, free fatty acids, and glycerol. Likewise, at rest and during maximal exercise, microcapillary arterialized blood was collected from the earlobe in order to determine erythrocyte 2,3-diphosphoglycerate, lactate, pH, K^+, $PaCO_2$, and components of arterial O_2 (CaO_2; i.e., arterial O_2 saturation [SaO_2], PaO_2, and hemoglobin). Details about the assays used are provided in the Table 2 footnote.

Mitochondrial Respiratory Capacity in Muscle Fibers

A sample of vastus lateralis muscle, obtained by using the percutaneous Bergstrom technique after applying local anesthesia (2% lidocaine), was immediately placed in an ice-cold solution that mimics intracellular fluid (20). The muscle fibers were separated under a binocular microscope and permeabilized with saponin (50 μg/mL) for 30 min, which allowed the sarcolemma to dissolve, but not the outer mitochondrial membrane. After being placed in a respiration buffer for 10 min (20) to wash out adenine nucleotides and creatine phosphate, skinned fibers were transferred in a 1-mL water-jacketed oxygraphic cell (Hansatech Instruments Ltd, Pentney, U.K.) equipped with a Clark electrode. Oxygen consumption (flux) reflects the first time derivative of the oxygen concentration in the respiration chambers, expressed as micromoles of O_2 per minute per gram of dry weight. Relative contributions of respiratory complexes I, II, and IV (CI, CII, CIV), and of oxidation/phosphorylation, were assessed by sequentially adding substrates/inhibitors: glutamate-malate (10:5 mmol/L), generating NADH,H^+ ($\dot{V}_{GM}$); the phosphate acceptor ADP (2 mmol/L) ($\dot{V}_{GM\text{-}ADP}$); the CI inhibitor rotenone (0.2 μmol/L); the electron donor for CII, succinate (25 mmol/L) ($\dot{V}_{Succ}$); the uncoupler carbonyl cyanide *m*-chloro phenyl hydrazone (CCCP; 1 μmol/L)

Table 2—Aerobic fitness, alveolar-capillary exchanges, and circulatory and metabolic data during incremental maximal exercise

	T1D (n = 16)	CON (n = 16)	P values (mixed-model main effects or Wilcoxon test)[a]
Aerobic fitness			
$\dot{V}o_{2max}$ (mL·min^{-1}·kg^{-1})	34.9 ± 7.2	40.7 ± 6.7	<0.05
Maximal aerobic power (W)	207.5 ± 41.9	230.0 ± 45.6	<0.05
Peak heart rate (bpm)	186.5 ± 10.6	188.4 ± 11.1	NS
O_2 pulse (mL · beat^{-1})			Exercise: <0.001, Group: NS, Interaction: NS
Rest	4.4 ± 1.5	5.2 ± 1.6	
Peak	14.5 ± 3.0	15.9 ± 3.8	
Peak RER	1.2 ± 0.1	1.1 ± 0.1	NS
Blood lactate at peak (mmol/L)	11.1 ± 3.0	11.4 ± 3.6	NS
Peak rate of perceived exertion	19.3 ± 0.9	18.7 ± 1.0	NS
Alveolar-capillary exchanges			
DL_{CO} (mL·min^{-1}·mmHg^{-1})[b]	31.8 ± 6.1	32.3 ± 5.8	NS
DL_{NO} (mL·min^{-1}·mmHg^{-1})	166.8 ± 25.7	174.0 ± 36.3	NS
D_m (mL·min^{-1}·mmHg^{-1})	84.6 ± 13.0	88.3 ± 18.4	NS
Vc (mL)	92.6 ± 22.9	95.3 ± 21.0	NS
Arterial O_2 transport			
PaO_2 (mmHg)			Exercise: <0.001, Group: <0.01, Interaction: NS
Rest	93.8 ± 6.1	99.7 ± 10.7	
Peak	99.5 ± 6.7†	110.9 ± 11.7††	
SaO_2 (%)			Exercise: <0.01, Group: NS, Interaction: NS
Rest	98.3 ± 1.1	98.5 ± 0.6	
Peak	97.4 ± 0.6	98.1 ± 0.8	
Hemoglobin (g·dL^{-1})			Exercise: <0.001, Group: NS, Interaction: <0.05
Rest	14.9 ± 1.2	15.0 ± 1.3	
Peak	16.7 ± 1.8††	15.5 ± 1.6	
CaO_2 (mL·100 mL^{-1})			Exercise: <0.001, Group: NS, Interaction: <0.05
Rest	20.4 ± 1.8	20.5 ± 1.7	
Peak	22.7 ± 2.3††	21.2 ± 2.2	
Factors able to shift the O_2-Hb dissociation curve and vasoactive substances			
pH			Exercise: <0.001, Group: NS, Interaction: NS
Rest	7.41 ± 0.04	7.42 ± 0.01	
Peak	7.26 ± 0.04	7.29 ± 0.08	
$PaCO_2$ (mmHg)			Exercise: <0.001, Group: NS, Interaction: NS
Rest	38.6 ± 2.4	37.5 ± 3.9	
Peak	31.3 ± 3.2	29.6 ± 4.3	
2,3-DPG (mmol·mL^{-1} red blood cells)			Exercise: NS, Group: <0.05, Interaction: NS
Rest	3.97 ± 0.89	3.45 ± 0.68	
Peak	4.11 ± 0.88	3.71 ± 0.70	
Serum free insulin (pmol·L^{-1})			Exercise: NS, Group: <0.001, Interaction: NS
Rest	344.4 ± 361.6	76.0 ± 40.2	
Peak	367.4 ± 442.0	73.2 ± 38.7	
Plasma epinephrine (pmol·L^{-1})			Exercise: <0.001, Group: NS, Interaction: NS
Rest	627.2 ± 425.4	561.8 ± 387.2	
Peak	1,499.8 ± 1,090.8	1,478.0 ± 796.3	
Plasma norepinephrine (pmol·L^{-1})			Exercise: <0.001, Group: NS, Interaction: NS
Rest	2,211.8 ± 922.6	2,726.3 ± 2,182.3	
Peak	11,260.3 ± 5,281.2	12,519.9 ± 4,743.0	

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Table 2—Continued

	T1D (n = 16)	CON (n = 16)	P values (mixed-model main effects or Wilcoxon test)[a]
Arterial K^+ (mmol·L^{-1})			Exercise: <0.001, Group: NS, Interaction: NS
Rest	4.9 ± 0.4	4.6 ± 0.5	
Peak	5.9 ± 1.0	5.4 ± 0.8	
Metabolic data			
Plasma glucose (mmol·L^{-1})			Exercise: <0.05, Group: <0.001, Interaction: NS
Rest	7.3 ± 2.9*	5.1 ± 0.6	
Peak	8.3 ± 2.1*	6.4 ± 0.8††	
Plasma free fatty acids (mmol·L^{-1})			Exercise: NS, Group: <0.05, Interaction: NS
Rest	0.297 ± 0.182	0.357 ± 0.190	
Peak	0.207 ± 0.104	0.350 ± 0.175	
Plasma glycerol (mg·L^{-1})			Exercise: <0.001, Group: <0.05, Interaction: NS
Rest	2.55 ± 1.37	2.64 ± 1.45	
Peak	5.44 ± 2.83††	7.86 ± 3.31†††	

Values are mean ± SD unless otherwise indicated. Plasma (fluorinated) glucose was measured with a hexokinase enzymatic assay on a modular automatic analyzer; serum free insulin, with a noncompetitive radioimmunoassay (Cisbio); plasma catecholamines (heparin, metabisulfite), with high-performance liquid chromatography; serum free fatty acids and glycerol, with colorimetric assays (reagents from Randox Laboratories); arterialized (a vasodilatory pomade was applied 5 min before) erythrocyte 2,3-diphosphoglycerate (2,3-DPG), with spectrophotometry (Sigma-Aldrich); arterialized pH, K^+, and $PaCO_2$, by potentiometry; SaO_2 and hemoglobin (Hb), by spectrophotometry; and PaO_2 and lactate, by amperometry on an ABL800 FLEX blood gas analyzer. Peak, at exhaustion from the incremental exercise; RER, respiratory exchange ratio; Rest, at rest just before the exercise. [a]Main effects from mixed models include P values for an exercise effect (Exercise), a group effect (Group), and an exercise × group interaction (Interaction). Post hoc analyses for a group effect found values were significantly different from those of the control subjects at *$P < 0.05$; post hoc analyses for a time effect found that values were significantly different from those at rest at †$P < 0.05$, ††$P < 0.01$, or †††$P < 0.001$. [b]Corrected by individual hemoglobin concentrations.

($\dot{V}_{Succ\text{-}CCCP}$); the CIII inhibitor antimycin A (2.5 μmol/L); and the artificial electron donor to cytochrome c, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine dihydrochloride (TMPD)–ascorbate (0.5:2.0 mmol/L). CIV capacity was assessed as an isolated step of the respiratory chain ($\dot{V}_{TMPD}$). The respiratory control ratio (RCR; $\dot{V}_{GM\text{-}ADP}/\dot{V}_{GM}$) was calculated as an index of coupling efficiency between oxidation (O_2 consumption) and phosphorylation (of ADP to ATP) with CI substrates. $\dot{V}_{Succ}/\dot{V}_{GM\text{-}ADP}$ and $\dot{V}_{TMPD}/\dot{V}_{GM\text{-}ADP}$ were calculated as internal normalizations in order to assess specific CII and CIV relative capacities independently of mitochondrial content. Mitochondrial capacities to oxidize carbohydrates and fatty acids were assessed in separate samples in the respiration buffer, in the presence of ADP (2 mmol/L) and malate (2 mmol/L), by sequentially adding pyruvate (1 mmol/L; $\dot{V}_{Pyr}$) and palmitoyl-carnitine (135 μmol/L; $\dot{V}_{PC}$). Maximal citrate synthase activity (expressed per milligrams of protein) was assessed by using spectrophotometric assay of muscle samples that had been immediately frozen in liquid nitrogen and preserved at −80°C.

Statistical Analyses

Statistical analyses were performed with IBM SPSS 19.0 software. Results are reported as the mean ± SD unless otherwise indicated. Nonrepeated data were compared between groups with the Wilcoxon matched-pairs test. Repeated data (normally distributed according to the Shapiro-Wilk test) were compared between groups (fixed effect) and according to exercise (fixed effect: rest vs. peak exercise or, for NIRS, ventilatory expiratory flow [$\dot{V}_E$], and O_2 pulse outcomes, relative exercise intensities with a value for every 10% $\dot{V}O_{2max}$, as well as absolute exercise intensities [Watts]) using linear mixed models for repeated measurements. If significant main effects or interactions were found, Bonferroni post hoc comparisons were applied. Correlations were tested using the Spearman ρ. $P < 0.05$ was considered statistically significant.

RESULTS

Demographic and anthropometric data did not differ between groups (Table 1). The T1D group had lower $\dot{V}O_{2max}$ (Table 2) than the CON group despite comparable levels of habitual physical activity (Table 1) and comparable heart rates at exhaustion. Plasma glucose increased during exercise in both groups, with overall higher values in the T1D group (Table 2). No hypoglycemia occurred during exercise in patients in the T1D group.

Alveolar-Capillary Diffusion

DL_{CO}, as well as its determinants D_m and $\dot{V}_c$, did not differ between groups (Table 2). $\dot{V}_c$ is influenced by the number of pulmonary capillaries in contact with ventilated alveoli, which increases during exercise because pulmonary blood flow and lung volumes increase. O_2 pulse (indirectly reflecting stroke volume) and $\dot{V}_E$ and its components (tidal volume, respiratory rate) increased throughout exercise, and we found no intergroup differences (in mixed models, the group effect and the exercise × group interaction were not significant; Table 2 for O_2 pulse; data not shown for V_E).

Oxygen Arterial Transport

Although PaO_2 was slightly lower in patients in the T1D group than in subjects in the CON group, CaO_2 and, correspondingly, Hb concentration were not impaired; these increased even more during exercise in the T1D group than in the CON group (Table 2).

Muscle Perfusion (ΔTHb) and O_2 Extraction (ΔHHb)

Despite higher serum insulin and normal catecholamine concentrations (Table 2), the levels of and increases in ΔTHb were lower among patients in the T1D group than among subjects in the CON group, especially at exercise intensities above

30% $\dot{V}O_{2max}$. The levels of and increases in ΔHHb were lower in the T1D group than in the CON group, particularly at exercise intensities above 50% $\dot{V}O_{2max}$. This occurred in spite of higher 2,3-diphosphoglycerate concentrations in the T1D group (Fig. 1 and Table 2).

Use of O_2 in Muscle Mitochondria

Citrate synthase activity was similar between the T1D and CON groups (92 ± 47 and 85 ± 31 pmol·min^{-1}·mg protein^{-1}, respectively). Oxygen flux with the mitochondrial substrates $\dot{V}_{GM\text{-}ADP}$ and $\dot{V}_{Pyr}$ (electrons through CI to CIII, CIV), $\dot{V}_{Succ}$ (electrons through CII to CIII, CIV), and $\dot{V}_{PC}$ (electrons through CI and CII to CIII, CIV) did not differ between groups (Fig. 2). The comparable $\dot{V}_{PC}$ and V_{Pyr}, measured in vitro in muscle, were in accordance with estimated in vivo whole-body lipid and carbohydrate oxidation rates throughout exercise (i.e., comparable $\dot{V}CO_2$ divided by $\dot{V}O_2$; data not shown). Alterations in neither ATP synthase ($\dot{V}_{Succ\text{-}CCCP}$ minus $\dot{V}_{Succ}$; data not shown), global electron transport system capacity (from CII: $\dot{V}_{Succ\text{-}CCCP}$), nor oxidation/phosphorylation coupling efficiency (RCR) were noticeable in patients in the T1D group. However, specific examination of the various mitochondrial chain complexes revealed impairment in the CIV relative capacity ($\dot{V}_{TMPD}$/$\dot{V}_{GM\text{-}ADP}$) in patients in the T1D group, whereas the CII relative capacity ($\dot{V}_{Succ}$/$\dot{V}_{GM\text{-}ADP}$) was unaltered.

It is noteworthy that in patients in the T1D group, diabetes duration correlated negatively with the CIV relative capacity ($\dot{V}_{TMPD}/\dot{V}_{GM\text{-}ADP}$; $r = -0.59$; $P < 0.05$), and HbA_{1c} tended to correlate negatively with CIV capacity ($\dot{V}_{TMPD}$; $r = -0.47$; $P < 0.07$). Moreover, longer diabetes duration and lower CIV relative capacity were predictors (covariates in mixed models) of smaller increases in ΔHHb with exercise intensity (interaction with exercise intensity, $e = -0.004$ [$P < 0.001$] and $e = +0.02$ [$P < 0.001$], respectively). The other steps of O_2 transport from the lungs to mitochondria were not significantly associated with HbA_{1c} or diabetes duration.

In the CON group, $\dot{V}_{TMPD}$ correlated with $\dot{V}O_{2max}$ and maximal aerobic power ($r > 0.64$; $P < 0.01$).

CONCLUSIONS

The novelty of this study resides in the examination of all steps of the pathway of oxygen, from air to mitochondria, by combining both in vivo and ex vivo approaches in patients with uncomplicated type 1 diabetes and strictly matched healthy control subjects (Supplementary Fig. 1). We showed that alveolar-capillary membrane diffusion capacity and arterial O_2 transport are normal at this stage of the disease. However, we confirmed that these patients display blunted perfusion and oxygen extraction in microvessels from active skeletal muscle at moderate to maximal exercise intensities. The defect in oxygen extraction occurred despite an overall normal intrinsic mitochondrial maximal respiratory capacity. The only detectable alteration in the mitochondrial chain appeared at the level of CIV, and this was more pronounced among patients with poorly controlled, long-standing diabetes.

Considering our results and the very few studies that matched their patient and control populations on physical activity levels (6,21), it seems that DL_{CO}, D_m, and $\dot{V}_c$ are not impaired and do not correlate with glycemic control in patients with uncomplicated type 1 diabetes. Thus, thickening of the pulmonary capillary basal lamina and dysfunction of the pulmonary vasculature are probably still absent, or are present but have no detectable consequences, when clinical complications are not overt. It is, however, still possible that some subtle alterations are already present in the participants with type 1 diabetes, as suggested by the reduced PaO_2. Notwithstanding, CaO_2 was adequately maintained throughout exercise in patients in the T1D group. Subtle alterations in lung function might have been balanced by a higher affinity for O_2 when hemoglobin is glycated (22), leading to normal SaO_2. It could also have been the case in a previous study in which patients with long-standing type 1 diabetes displayed altered DL_{CO} and D_m in a more demanding situation (intense exercise in a hypoxic environment) without any repercussions for SaO_2 (6).

Although the first steps of oxygen supply that occur in the lungs and arteries resulted in normal arterial O_2 transport throughout exercise in patients in the T1D group, the subsequent steps in muscle microvessels seemed to be impaired. Very few studies have investigated exercise-induced muscle vasoreactivity in type 1 diabetes. Rissanen et al. (23) reported reduced muscle blood flow in the active leg of adults with type 1 diabetes; this reduced flow occurred at peak intensity during an incremental cycling exercise and was measured by using an indirect method based on deoxygenation patterns (%ΔHHb) and theoretical values of peripheral arterial-venous O_2 difference. In this work and in our previous study (8), we also highlighted an impaired exercise-induced increase in muscle blood volume in response to maximal incremental exercise.

Admittedly, cardiac output is one determinant of muscle perfusion (24). We used an indirect marker of stroke volume, which seemed to be normal throughout exercise in patients in the T1D group. The available literature related to cardiac output in uncomplicated patients with type 1 diabetes and physical activity–matched control subjects conflicts: one report highlights low cardiac output during submaximal exercise in adolescents (25), whereas others describe no intergroup differences in adults at submaximal (23) or peak (6,10) exercise. Even if the involvement of central cardiovascular factors cannot be totally disregarded in relation to lower muscle blood volume, the latter is probably also triggered by peripheral microvascular alterations. Supporting this hypothesis, Pichler et al. (26) found an impaired increase in blood flow in the brachioradialis muscle in children with type 1 diabetes during the recovery period after a short rhythmic handgrip test, a local exercise involving a very small muscle mass and wherein muscle blood flow is unlikely to be limited by cardiac output.

Following the few previous studies of exercise-induced muscle microvascular reactivity limits in type 1 diabetes (8,23), here we go a step further into understanding putative underpinning mechanisms. Although their relative contributions remain controversial, it is well recognized that arterial, capillary, and venous compartments all participate in the microvascular THb signal in muscle (19). Within muscle arterioles, although norepinephrine is a vasoconstrictor devoted to regulating blood pressure, epinephrine, as well as insulin (27), can promote endothelial nitric oxide production and hence vasorelaxation. In our study, although plasma catecholamines

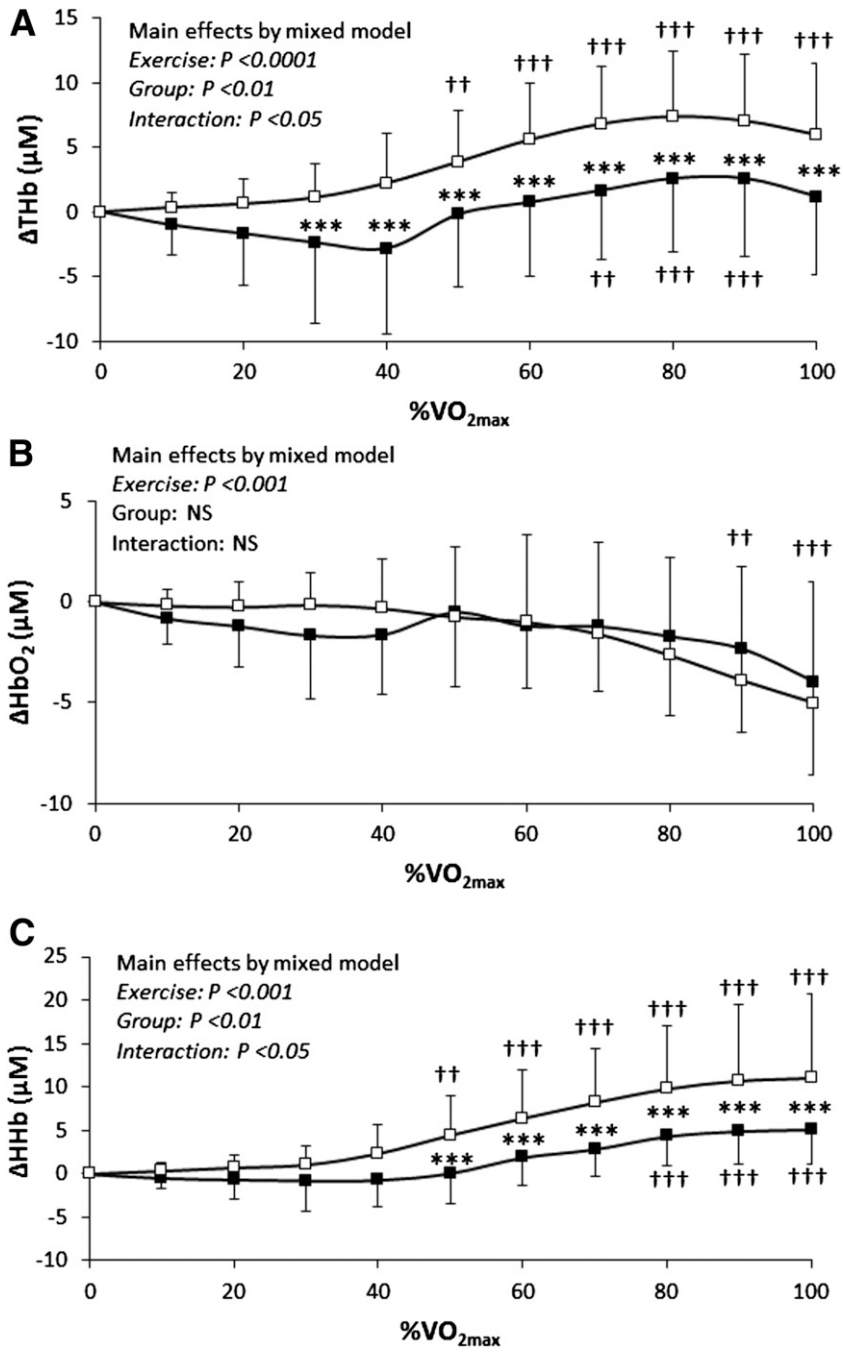

Figure 1—Recordings from NIRS of vastus lateralis, showing changes in ΔTHb (*A*), ΔO_2Hb (*B*), and ΔHHb (*C*). The 40-mm interspersed emitter-detector pair was placed on the belly of the right vastus lateralis muscle. Changes in NIRS outcomes are displayed according to exercise intensities expressed as relative values ($\%\dot{V}O_{2max}$). Mixed models revealed almost identical results when studying NIRS according to absolute exercise intensities (i.e., expressed as absolute work rates in Watts; data not shown). Values are the mean ± SE. ■, patients in the TID group; □, healthy subjects in the CON group. Post hoc analyses for the group effect were significantly different from control subjects at ***$P < 0.001$. Post hoc analyses for time effect were significantly different from those at rest at ††$P < 0.01$ and †††$P < 0.001$.

were comparable, serum free insulin was markedly elevated in patients in the T1D group. The high insulin concentrations, concomitant with high plasma glucose levels, reflect the well-described state of insulin resistance in type 1 diabetes, which could presumably also apply at the endothelial cell level, as already proven in obesity and type 2 diabetes (27). This might explain the impaired muscle vasoreactivity despite the higher insulin concentrations observed in the T1D group. Of note, the higher plasma glucose levels observed throughout exercise in the T1D group can certainly not explain the concomitant smaller increases in regional blood volume. Dye et al. (28) indeed found an inverse effect with increased postocclusive reactive hyperemia-induced vasodilatation under hyperglycemic conditions (200 $mg \cdot dL^{-1}$ glucose) in patients with type 1 diabetes. The other vasoactive moieties that we considered ($PaCO_2$, K+, pH) did not significantly differ between the two groups. Although muscle microvascular density is presumably normal in patients with uncomplicated type 1 diabetes (10,16), further studies are needed to fully determine the molecular mechanisms of the reduced exercise-induced muscle vasoreactivity. Although Fayh et al. (29) hypothesized that nitric oxide production is not involved in the low blood flow after exercise that they observed in young adults with type 1 diabetes, this result is worth confirming, as they did not distinguish nitrates from nitrites in their measurements. Only nitrites are known to sensitively reflect acute changes in nitric oxide synthase activity (30).

Because muscle O_2 and high-energy phosphate stores are small, any sustained elevation in ATP turnover in active skeletal muscle during exercise requires that the rate of O_2 delivery to muscle mitochondria precisely match the muscle's O_2 requirements. In this study, the last step of the delivery of oxygen to skeletal muscle before its utilization, that is, muscle O_2 extraction (ΔHHb), was significantly blunted in response to increased exercise intensity in the T1D group. This was more pronounced in the case of long-standing diabetes, and it occurred despite higher erythrocyte 2,3-diphosphoglycerate concentrations. As previously suggested (8), this result, obtained from a sample with rather poorly controlled diabetes, could be partly explained by an impairment of oxyhemoglobin dissociation near active skeletal muscle, induced by the greater affinity of HbA_{1c} for O_2 than that of nonglycated hemoglobin for O_2 (22). It is noteworthy that adjustments in 2,3-diphosphoglycerate concentrations compensatory to increased HbA_{1c} formation in type 1 diabetes may be insufficient to maintain normal erythrocyte O_2 dissociation (31).

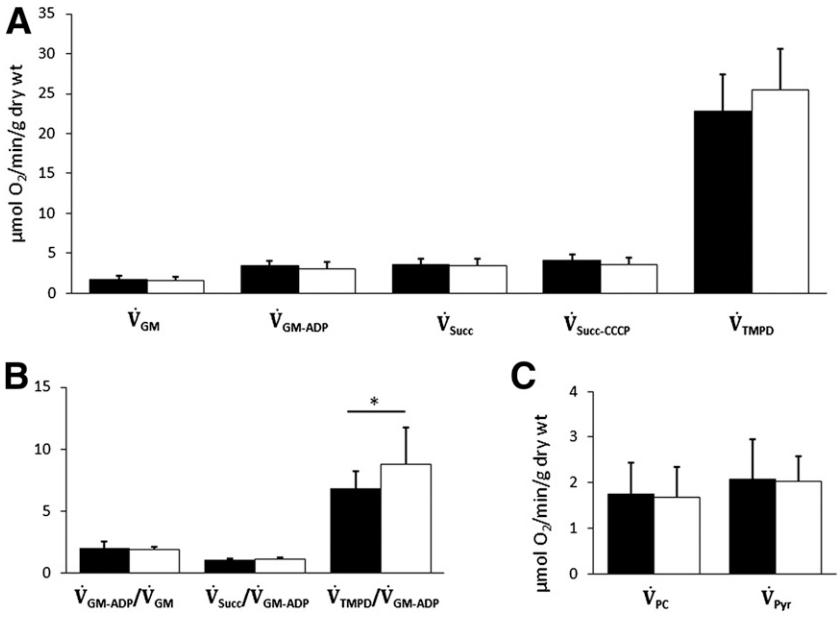

Figure 2—Mitochondrial respiratory capacity in saponin skinned fibers of the vastus lateralis muscle. *A*: Step-by-step analysis of various segments of the respiratory chain. $\dot{V}_{GM}$, basal adenylate-free leak CI respiration: O_2 flux with glutamate–malate (10:5 mmol/L) to assess O_2 spent in order to maintain the membrane potential, with electron flow from malate dehydrogenase–produced $NADH,H^+$ going though CI, CIII, and CIV, that is, respiration due to protons leaking and slipping back into the mitochondrial matrix, cation cycling, and, to a small extent, an electron leak induced by reactive oxygen species production (malate at 2 mmol/L is used to initiate the Krebs cycle while redirecting 2-oxoglutarate outside of the mitochondria, instead of producing succinate). $\dot{V}_{GM\text{-}ADP,}$ oxidative phosphorylation (OXPHOS) capacity from CI (through CIII and CIV): O_2 flux after the phosphate acceptor ADP was added at a saturating concentration (2 mmol/L). $\dot{V}_{Succ}$, OXPHOS capacity from CII (through CIII and CIV): O_2 flux after the CI inhibitor rotenone (0.2 µmol/L) and then the electron donor for complex II, succinate (25 mmol/L), were sequentially added in order to assess mitochondrial respiration from $FADH_2$. $\dot{V}_{Succ\text{-}CCCP}$, global electron transport system capacity from CII and capacity of succinate dehydrogenase: O_2 flux after the uncoupler CCCP (1 µmol/L) was added, thereby bypassing the control of the phosphorylation system, in order to assess uncoupled O_2 flux with an electron supply from $FADH_2$. $\dot{V}_{TMPD}$, CIV capacity: O_2 flux after the CIII inhibitor antimycin A (2.5 µmol/L) and then TMPD–ascorbate (0.5:2 mmol/L, as an artificial electron donor to cytochrom c) were sequentially added. *B*: RCRs. $\dot{V}_{GM\text{-}ADP}/\dot{V}_{GM}$, oxidation-phosphorylation coupling efficiency (RCR): ratio of the respiration rate before and the respiration rate after a saturating concentration of ADP was added. $\dot{V}_{Succ}/\dot{V}_{GM\text{-}ADP}$: specific CII relative capacities. $\dot{V}_{TMPD}/\dot{V}_{GM\text{-}ADP}$: specific CIV relative capacities. *C*: Mitochondrial capacities to oxidize carbohydrates and fatty acids. $\dot{V}_{Pyr}$, mitochondrial capacity to oxidize carbohydrates. $\dot{V}_{PC}$: mitochondrial capacity to oxidize fatty acids. These were assessed in separate samples in the respiration buffer in the presence of ADP (2 mmol/L) and malate (2 mmol/L). Pyruvate (1 mmol/L; an index of glucose oxidation) and palmitoyl-carnitine (135 µmol/L; undergoing β-oxidation) were added sequentially. All respiratory measurements were performed simultaneously, in duplicate, at 22°C under continuous stirring, and in the presence of saturating concentrations of the substrates at an initial O_2 concentration of ~220 µmol/L. Black bars represent patients with type 1 diabetes; white bars represent the healthy control subjects. Results are the mean ± SD. *$P < 0.05$ vs. the control subjects.

Besides the putative reduced oxyhemoglobin dissociation, the influence of impaired mitochondrial O_2 use on the blunted ΔHHb signal cannot be ruled out. To clarify the partition between both mechanisms, we combined, through an integrated approach, an ex vivo analysis of muscle biopsies with in vivo exploration of O_2 extraction during exercise. In the T1D group, no major alteration occurred in mitochondrial oxidative capacity. Mitochondrial content (citrate synthase activity) was comparable in both groups (T1D and CON), as previously suggested (12,13). We also found normal overall intrinsic mitochondrial maximal respiratory capacity with the various mitochondrial substrates. Of note, although the ex vivo intrinsic capacity of mitochondria to oxidize palmitate and the in vivo free fatty acid oxidation rate were normal in patients in the T1D group, lipolysis might be blunted, as suggested by the lower amount of circulating glycerol at rest and during maximal exercise. The latter observation must be considered in conjunction with the concomitant higher circulating insulin among patients in the T1D group, because insulin is a potent inhibitor of lipolysis.

To date, to our knowledge only one other research group (16) has provided insight into mitochondrial oxidative capacities in functional intact mitochondria under in situ–like conditions in type 1 diabetes. In line with our results, albeit in a smaller sample of subjects (11 patients and 8 healthy subjects), Monaco et al. (16) did not observe any alteration in CI-supported mitochondrial respiration, regardless of the substrate used. However, in contrast to our results and to those of previous studies examining isolated maximal capacity of succinate dehydrogenase (12,14), Monaco et al. observed a lower capacity for CII-supported respiration by succinate. In the latter study, BMI was higher in the patients with type 1 diabetes than in the healthy control subjects. This intergroup difference might partly explain the discordance with our results about CII-supported respiration capacity. Diet-induced obesity in animals has indeed been shown to decrease the rate of CII substrate–driven ATP synthesis in cardiac muscle (32), and weight loss in obese humans is associated with improvement in CII activity in adipose tissue (33). Accordingly, by further testing correlations between participant characteristics and mitochondrial respiration among patients with type 1 diabetes, we found that fat mass percentage, as objectively measured by DEXA, inversely correlated with $\dot{V}_{Succ}$ ($r = -0.51$; $P < 0.05$).

CIV of the electron transport chain was not specifically investigated by Monaco et al. (16), although it represents a main site for mitochondrial diseases (34). CIV is the terminal component of the mitochondrial respiratory chain and is essential for mitochondrial energy transduction. It catalyzes electron transfer from cytochrome c to molecular oxygen, generating a proton gradient required for ATP synthesis. Strikingly, the relative contribution of CIV was significantly reduced (by ~29%) in patients with type 1 diabetes in our study—mostly in those with longer diabetes duration and higher HbA_{1c} levels. The underlying mechanisms of such an impairment remain to be investigated, but

chronic hyperglycemia-induced oxidative stress may be part of the picture. It is well known that excessive glucose provision to mitochondria elevates production of reactive oxygen species (3), and as demonstrated in bovine heart muscle, CIV represents an important target for oxidative damage (35), thereby contributing to mitochondrial dysfunction (36). Particularly in type 1 diabetes, the low insulin concentration in the portal circulation, due to the peripheral mode of insulin administration, shifts glucose metabolism to produce excessive hepatic glucose, while skeletal muscle is forced to accept the high glucose load in a context of high peripheral circulating insulin (37).

Studies closely mimicking in vivo conditions by using saponin-permeabilized human muscle fibers have demonstrated that CIV exerts tight control over respiration, with only a low excess capacity of cytochrome oxidase. This tight control by CIV is even more pronounced in cases of low physiological oxygen concentrations (38), which can explain the pathological phenotype of mild cytochrome c oxidase deficiencies in mitochondrial myopathies (34). Consistent with this, the relative CIV capacity defect in patients in the T1D group in our study may have implications for aerobic fitness: the lower CIV capacity significantly predicted the blunted exercise-induced increase in muscle O_2 extraction in the T1D group, whereas higher CIV capacity was associated with higher aerobic fitness in the CON group.

Last, although changes in skeletal muscle have been intensively studied in rodent models of type 1 diabetes (39), further investigation in humans is required in order to supplement our mechanistic understanding of observed mitochondrial dysfunctions. Rodent models of diabetes cannot be directly transposed to humans because tight blood glucose control through multiple insulin injections is virtually impossible to achieve over long periods of time in animals. In particular, assessing supramolecular interactions of CIV with other complexes might be of great value. The structural and functional organization of the electron transport chain could indeed change from freely moving structures to assembled ones called supercomplexes, which are believed to increase transport efficiency and limit the production of reactive oxygen species. In a mouse model of type 1 diabetes mellitus, overexpression of mitofilin, a protein that affects supercomplex assembly, was able to restore mitochondrial function (40).

In summary, maximal aerobic exercise could represent a physiological way to identify possible subclinical defects in the serial steps responsible for appropriately adjusting O_2 delivery and subsequently utilizing O_2 in mitochondria. This investigation revealed that relatively young patients with type 1 diabetes display blunted muscle microvascular reactivity to exercise along with a lower relative capacity of CIV in the mitochondrial respiratory chain. Early microvascular and muscle oxidative capacity dysregulations, in addition to their negative effects on aerobic fitness—a strong predictor of cardiovascular risk—could also have deep long-term consequences on the primary determinants of diabetes complications. Defects in blood and nutrient delivery to skeletal muscle, as well as altered subsequent mitochondrial oxidation, can indeed directly affect glycemic and lipid profiles. Skeletal muscle is actually known to be responsible for most insulin-stimulated whole-body glucose disposal and for roughly half of non-insulin-mediated glucose uptake in the presence of hyperglycemia (41). It is also quantitatively the most dominant tissue with respect to lipid metabolism. In the face of these defects, implementing nonpharmacological interventions such as specific exercise training programs might be of the utmost clinical importance, especially because skeletal muscle is a highly malleable tissue with the capacity to metabolically adapt in response to contractile activity. The challenge of future studies will be to ensure that these defects in peripheral tissue perfusion—observed at even light to moderate exercise intensities—could be improved by training and euglycemia.

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