Muscle Oxygen Supply Impairment during Exercise in Poorly Controlled Type 1 Diabetes

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ABSTRACT

TAGOUGUI, S., E. LECLAIR, P. FONTAINE, R. MATRAN, G. MARAIS, J. AUCOUTURIER, A. DESCATOIRE, A. VAMBERGUE, K. OUSSAIDENE, G. BAQUET, and E. HEYMAN. Muscle Oxygen Supply Impairment during Exercise in Poorly Controlled Type 1 Diabetes. *Med. Sci. Sports Exerc.*, Vol. 47, No. 2, pp. 231–239, 2015. **Purpose**: Aerobic fitness, as reflected by maximal oxygen (O_2) uptake ($\dot{V}O_{2max}$), is impaired in poorly controlled patients with type 1 diabetes. The mechanisms underlying this impairment remain to be explored. This study sought to investigate whether type 1 diabetes and high levels of glycated hemoglobin (HbA_{1c}) influence O_2 supply including O_2 delivery and release to active muscles during maximal exercise. **Methods**: Two groups of patients with uncomplicated type 1 diabetes (T1D-A, $n = 11$, with adequate glycemic control, HbA_{1c} <7.0%; T1D-I, $n = 12$ with inadequate glycemic control, HbA_{1c} >8%) were compared with healthy controls (CON-A, $n = 11$; CON-I, $n = 12$, respectively) matched for physical activity and body composition. Subjects performed exhaustive incremental exercise to determine $\dot{V}O_{2max}$. Throughout the exercise, near-infrared spectroscopy allowed investigation of changes in oxyhemoglobin, deoxyhemoglobin, and total hemoglobin in the vastus lateralis. Venous and arterialized capillary blood was sampled during exercise to assess arterial O_2 transport and factors able to shift the oxyhemoglobin dissociation curve. **Results**: Arterial O_2 content was comparable between groups. However, changes in total hemoglobin (i.e., muscle blood volume) was significantly lower in T1D-I compared with that in CON-I. T1D-I also had impaired changes in deoxyhemoglobin levels and increase during high-intensity exercise despite normal erythrocyte 2,3-diphosphoglycerate levels. Finally, $\dot{V}O_{2max}$ was lower in T1D-I compared with that in CON-I. No differences were observed between T1D-A and CON-A. **Conclusions**: Poorly controlled patients displayed lower $\dot{V}O_{2max}$ and blunted muscle deoxyhemoglobin increase. The latter supports the hypotheses of increase in O_2 affinity induced by hemoglobin glycation and/or of a disturbed balance between nutritive and nonnutritive muscle blood flow. Furthermore, reduced exercise muscle blood volume in poorly controlled patients may warn clinicians of microvascular dysfunction occurring even before overt microangiopathy. **Key Words**: AEROBIC FITNESS, GLYCATED HEMOGLOBIN, OXYGEN DELIVERY, OXYGEN RELEASE, SKELETAL MUSCLE

The beneficial effects of physical activity and the advantages of good physical fitness are well established both in healthy individuals and in those with chronic disease (20,21). Aerobic fitness, as measured by maximal oxygen (O_2) uptake ($\dot{V}O_{2max}$), is a strong predictor of cardiovascular risk (2). In patients with type 1 diabetes, poor fitness represents an important barrier to regular physical activity (9). Consequently, better understanding of the underlying factors involved in the possible impairment of $\dot{V}O_{2max}$ in patients with type 1 diabetes is warranted.

Low aerobic fitness levels have been reported in several (28,32), albeit not all (21,39), studies in adults with type 1 diabetes and seem to be associated with poor glycemic control, as reflected by high glycated hemoglobin (HbA_{1c}) levels (5,24,30). A high HbA_{1c} level is indeed an important factor in the initiation and progression of micro- and macrovascular complications. In turn, these complications may alter the functioning of tissues that are important for exercise adaptations, such as blood vessels, lungs, and heart (11,41) and, consequently, reduce $\dot{V}O_{2max}$.

However, there may be other factors involved in the impairment of aerobic fitness observed in individuals with poor glycemic control. In this respect, the HbA_{1c}– $\dot{V}O_{2max}$ relation has been found even in the absence of diabetic complications in young patients with type 1 diabetes (24,35). O_2 supply, including arterial O_2 delivery and O_2 release (i.e., oxyhemoglobin (O_2Hb) dissociation) to active muscles, is a well-established factor influencing $\dot{V}O_{2max}$ in healthy subjects or subjects with a chronic disease (17,29). In adolescents with type 1 diabetes, one study reported reduction in forearm blood flow after local exercise (rhythmic handgrip) despite the absence of any otherwise clinically detectable vascular disorders (33). To our knowledge, arterial O_2 transport capacity has never been investigated during exercise in patients with type 1 diabetes and with different levels of HbA_{1c}.

Several *in vitro* studies suggest possibly increased O_2Hb affinity (i.e., a leftward shift of the O_2 dissociation curve) and/or decreased sensitivity of hemoglobin to the allosteric effect of organic phosphates as a result of hemoglobin glycation at a degree corresponding to HbA_{1c} levels in patients with poor glycemic control (i.e., 8%) (10,25,26,36). Some further insight into muscle O_2Hb dissociation in type 1 diabetes is required, particularly *in vivo* during maximal exercise and in accordance with HbA_{1c} levels. In this respect, near-infrared spectroscopy (NIRS) allows noninvasive and functional monitoring of changes in skeletal muscle oxygenation including O_2Hb dissociation (18). Besides hemoglobin glycation, it is important to take into account the erythrocyte 2,3-diphosphoglycerate (2,3-DPG) level. This stimulus for a rightward shift of the O_2Hb dissociation

curve has been found to be, contradictorily, either reduced (15,16) or elevated in patients with type 1 diabetes (8), especially in cases of chronically impaired glycemic control (1).

Therefore, we aimed to determine whether O_2 delivery and/or release to an active muscle during maximal exercise is altered in patients with uncomplicated type 1 diabetes and high levels of HbA_{1c} and whether any subsequent relation to impairment in $\dot{V}O_{2max}$ exists.

MATERIAL AND METHODS

A written informed consent was obtained from all participants before their inclusion in this study, which was approved by the North Western IV regional ethics committee (N° EudraCT, 2009-A00746-51). Twenty-three patients, age 18–40 yr, who had type 1 diabetes for at least 1 yr and were free from vascular complications, volunteered to participate in this study (Table 1). The absence of microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular (high blood pressure, coronary disease, peripheral arteriopathy) complications was carefully checked by a clinician during the initial examination. The patients were then divided into two groups according to their HbA_{1c} levels measured at inclusion, as follows: adequate glycemic control, T1D-A ($n = 11$; HbA_{1c} <7% (53 mmol·mol^{-1})); and inadequate glycemic control, T1D-I ($n = 12$; HbA_{1c} >8% (64 mmol·mol^{-1})). Two control groups, CON-A and CON-I, composed of healthy subjects age 18–40 yr, were recruited (as described in the following section) to strictly match the T1D-A and T1D-I groups, respectively.

TABLE 1. Participants' characteristics.

	CON-A	T1D-A	CON-I	T1D-I
Anthropometric and demographic data				
Total (male/female), *n*	11 (11/0)	11 (11/0)	12 (7/5)	12 (7/5)
Age (yr)	25.9 ± 5.6	27.1 ± 6.1	26.2 ± 5.0	25.5 ± 7.3
Smoking status (smokers/nonsmokers)	1/10	1/10	2/10	2/10
BMI (kg·m^{-2})	23.5 ± 2.5	23.4 ± 3.0	22.9 ± 1.8	23.1 ± 1.8
Fat mass (%)	15.5 ± 3.8	16.7 ± 5.5	19.6 ± 5.8	20.0 ± 7.5
Right leg fat mass (%)	20.5 ± 7.8	20.9 ± 7.7	23.8 ± 9.0	23.6 ± 9.7
Right leg lean mass (kg)	9.78 ± 1.86	9.74 ± 1.37	9.98 ± 1.62	9.14 ± 2.48
Adipose thickness at the right vastus lateralis (mm)	10.67 ± 2.32	9.86 ± 3.19	11.57 ± 2.52	10.27 ± 2.76
HbA_{1c} (%)	5.5 ± 0.2	6.6 ± 0.7*	5.3 ± 0.2	9.1 ± 0.7**
HbA_{1c} (mmol·mol^{-1})	37.0 ± 2.2	49.0 ± 7.7*	34.8 ± 2.2	76.1 ± 7.7**
Diabetes duration (yr)		4.5 ± 3.6		10.9 ± 3.4
Insulin delivery (MDI/CSII)		9/2		8/4
Accelerometry (min·d^{-1})				
Light + moderate + vigorous intensities	182.5 ± 49.9	189.4 ± 76.2	233.4 ± 70.2	207.1 ± 76.5
Usual daily macronutrient intake				
TC (kJ·kg^{-1}·d^{-1})	143.3 ± 34.1	134.5 ± 32.2	123.6 ± 30.6	120.4 ± 26.3
Protein (% TC)	16.1 ± 3.3	16.3 ± 2.3	15.5 ± 3.6	14.9 ± 2.6
Fat (% of TC)	34.5 ± 4.7	33.8 ± 4.2	34.7 ± 4.0	32.9 ± 8.4
Polyunsaturated/saturated fatty acids ratio	0.4 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.2
Cholesterol (mg·d^{-1})	303.7 ± 135.4	363.6 ± 142.5	286.6 ± 148.8	287.5 ± 122.6
CHO (% of TC)	45.8 ± 7.2	47.0 ± 3.6	46.6 ± 5.5	48.6 ± 7.6
High glycemic index CHO (% of TC)	19.8 ± 4.2	15.1 ± 5.5	17.3 ± 4.9	16.7 ± 5.1
Fiber intake (g·d^{-1})	18.9 ± 4.9	21.3 ± 4.9	17.7 ± 4.9	19.2 ± 4.8

Values are means ± SD. Fat mass was measured by dual energy x-ray absorptiometry. HbA_{1c} was recorded just before exercise.
*Significantly different from their respective CON group (Wilcoxon test), $P < 0.01$.
**Significantly different from their respective CON group (Wilcoxon test), $P < 0.001$.
BMI, body mass index; CSII: continuous subcutaneous insulin infusion; MDI, multiple daily injections; TC, total caloric intake.

Selection process of the healthy control subjects.

Healthy participants were selected from a list ($n = 250$) drawn from patients' friends and contacts. Each healthy control was chosen to strictly match a patient with type 1 diabetes according to the following preestablished ranges or values: gender, same as the case patient; age, ±7 yr; body mass index, ±4 kg·m^{-2}; moderate-to-vigorous physical activity levels, ±1 h when the patients' physical activity category was 0 h·wk^{-1}, ±2 h for category 2–6 h·wk^{-1}, ±4 h for category >6 h·wk^{-1}, pairs of patient/control being in the same category, and tobacco status, grouped according to no smoking, <10 cigarettes a day, and >10 cigarettes a day. The healthy controls chosen were then recruited after an oral glucose tolerance test (75 g). Individuals were excluded if they had a fasting blood glucose level of >6.05 mM or an abnormal glucose tolerance test result using World Health Organization criteria (14). The similarity of body composition and physical activity levels between groups was then accurately checked using dual energy x-ray absorptiometry (Hologic, Inc., Waltham, MA) and accelerometry (ActiGraph, model GT1M) over seven consecutive days, respectively (Table 1).

Besides physical activity levels, we added an assessment of lifestyle diet. Dietary data were based on a 3-d diary (based on two weekdays and one weekend day). Written instructions were given to provide detailed information about the quantity and quality of all items consumed. The patients were then interviewed by a research-trained dietitian, who gathered information to supplement the diaries.

Laboratory testing.

Subjects were requested to refrain from vigorous physical activity for 48 h before the test and from using tobacco in the morning of the test. Patients with type 1 diabetes received their usual morning insulin bolus, and all subjects consumed a breakfast (providing 8.5% $\pm$ 3.4% proteins, 41.4% $\pm$ 16.4% lipids, and 50.1% $\pm$ 15.8% CHO) based on their usual breakfast and previously agreed upon by a dietician. The exercise test began 3.8 ± 0.6 h after breakfast. After a 2-min resting period sitting on the cycle ergometer (Excalibur Sport; Lode BV, Groningen, Netherlands), the test started at 30 W and continued with a 20-W increment every 2 min until exhaustion.

Cardiopulmonary response.

An ECG (Ergocard®; Medisoft, Dinant, Belgium) was performed at rest and continually monitored throughout the exercise test by a cardiologist.

Pulmonary gas exchanges were measured continuously throughout exercise (breath-by-breath system, Ergocard®). $\dot{V}O_{2max}$ was determined as the highest 15-s average value during the exercise test. Validation of $\dot{V}O_{2max}$ was obtained at the termination of the test when three of the following five criteria were attained: 1) $\dot{V}O_2$ increase of <100 mL·min^{-1} with the 20-W increase in power output, 2) an HR >90% of the theoretical HR$_{max}$ ($210 - 0.65 \times$ age), 3) an RPE score ≥19, 4) blood lactate >8 mM, and 5) an RER >1.1 (4). According to these criteria, all subjects achieved their $\dot{V}O_{2max}$ (Table 2).

The O_2 pulse (mL·beat^{-1}) was calculated as the ratio between O_2 consumption and HR and was used as an indicator of stroke volume during submaximal and maximal exercise (40).

NIRS measurements.

Subjects were equipped with an NIRS probe to monitor the absorption of light across muscle tissues throughout the exercise test (Oxymon Mk III; Artinis Medical Systems, Zetten, The Netherlands). The emitter-and-detector pair was placed on the belly of the right vastus lateralis muscle, midway between the lateral epicondyle and greater trochanter of the femur. The vastus lateralis muscle is highly active during cycling (23), making it suitable for examining exercise-induced changes in active muscle oxygenation. Adipose tissue thickness has been reported to have substantial confounding influence on *in vivo* NIRS measurements (18). Therefore, we ensured that the vastus lateralis skinfold was <1.5 cm and that fat mass percentage in the right leg was comparable between groups (Table 1). The interoptode distance was 40 mm. NIRS data were collected with a sampling frequency of 10 Hz.

The Beer–Lambert law was used to calculate changes in tissue oxygenation O_2Hb (ΔO_2Hb) and deoxyhemoglobin (ΔHHb) across time using optical densities from two continuous wavelengths of NIR light (780 and 850 nm). The change in total hemoglobin (ΔTHb), i.e., the arithmetic sum of ΔO_2Hb and ΔHHb, was used as an index of change in regional blood volume (19). ΔHHb was used as a sensitive reflection of relative tissue deoxygenation due to O_2 extraction because this measure is closely associated with changes in venous O_2 content and is less sensitive to ΔTHb than ΔO_2Hb (19). NIRS measurements were normalized to reflect changes from the 1-min baseline period immediately before beginning the exercise protocol (arbitrarily defined as 0 μM) to express the magnitudes of changes throughout exercise.

The use and limitations of NIRS have been extensively reviewed (7,18). Noteworthy, the NIRS technique is unable to differentiate between the amount of O_2 released by both hemoglobin and myoglobin because the absorbency signals of these two chromophores overlap in the NIR range. On the basis of data available on hemoglobin/myoglobin ratio in human muscles, one can estimate that myoglobin represents a confounding factor of 10% of the whole hemoglobin signal (18). Consequently, it can be assumed that most of the NIRS signal reflects changes in absorption of ΔO_2Hb and ΔHHb (18).

Blood analyses.

Venous blood samples were collected from a forearm catheter at rest and at maximal exercise. HbA_{1c} was measured at rest from EDTA blood (HPLC assay, VARIANT^2 Turbo; Bio-Rad) (Table 1). At rest and maximal exercise, fluorinated and heparinized samples were used to analyze blood glucose (hexokinase enzymatic assay, Modular automat) and erythrocyte 2,3-DPG (spectrophotometry; Sigma-Aldrich, St. Louis, MO), respectively.

At rest and immediately at exhaustion, a microcapillary arterialized earlobe blood sample (vasodilator pomade

TABLE 2. Cardiopulmonary and metabolic data from participants during incremental maximal exercise.

	CON-A	T1D-A	Main Effect by ANOVA	CON-I	T1D-I	Main Effect by ANOVA
Aerobic fitness						
$\dot{V}O_{2max}$ (mL·min^{-1}·kg^{-1})	43.0 ± 7.8	40.9 ± 9.3		41.2 ± 7.2	34.6 ± 7.2[‡]	
HR$_{max}$ (bpm)	190.6 ± 8.8	191.6 ± 12.3		189.4 ± 11.5	185.9 ± 12.2	
RER$_{max}$	1.1 ± 0.1	1.1 ± 0.1		1.1 ± 0.1	1.2 ± 0.1[‡]	
Blood lactate max (mM)	13.6 ± 4.7	12.7 ± 2.5		11.9 ± 4.2	13.6 ± 2.2	
RPE max	19.1 ± 0.8	18.8 ± 0.9		18.8 ± 0.6	19.1 ± 0.9	
O$_2$ pulse (mL·beat^{-1})						
Rest	4.9 ± 1.7	4.5 ± 1.6	Exercise, $P < 0.001$	4.7 ± 1.7	4.8 ± 2.1	Exercise, $P < 0.001$
Max	16.6 ± 3.1	16.0 ± 3.4	Group, NS Interaction, NS	15.1 ± 3.7	13.6 ± 3.7	Group, NS Interaction, NS
Arterial O$_2$ transport						
PaO$_2$ (mm Hg)						
Rest	97.6 ± 8.2	97.9 ± 13.5	Exercise, $P < 0.01$ Group, NS	98.7 ± 9.7	90.9 ± 7.6	Exercise, $P < 0.01$ Group, NS
Max	107.0 ± 13.2[†]	102.4 ± 12.9	Interaction, NS	105.8 ± 6.9[†]	103.4 ± 14.2[†††]	Interaction, NS
SaO$_2$ (%)						
Rest	97.9 ± 0.6	98.2 ± 1.0	Exercise, $P < 0.05$ Group, NS	98.5 ± 0.5	97.9 ± 1.3	Exercise, $P < 0.01$ Group, NS
Max	97.2 ± 1.8	97.0 ± 1.1[†]	Interaction, NS	97.3 ± 0.6[††]	97.4 ± 0.7	Interaction, NS
Hemoglobin concentration (g·dL^{-1})						
Rest	15.0 ± 0.7	15.8 ± 1.1	Exercise, $P < 0.0001$ Group, $P < 0.05$	14.0 ± 1.7	14.6 ± 1.4	Exercise, $P < 0.01$ Group, NS
Max	16.0 ± 0.8	17.3 ± 1.9	Interaction, NS	15.0 ± 1.9	15.8 ± 1.3[†]	Interaction, NS
CaO$_2$ (mL per 100 mL)						
Rest	20.4 ± 1.0	21.6 ± 1.6	Exercise, $P < 0.0001$ Group, $P < 0.05$	19.2 ± 2.3	19.9 ± 2.0	Exercise, < 0.01 Group, NS
Max	21.8 ± 1.2	23.4 ± 2.6[†]	Interaction, NS	20.3 ± 2.4	21.4 ± 1.7[†]	Interaction, NS
Factors influencing O$_2$Hb dissociation curve						
pH						
Rest	7.41 ± 0.02	7.40 ± 0.04	Exercise, $P < 0.0001$ Group, NS	7.42 ± 0.02	7.42 ± 0.01	Exercise, $P < 0.0001$ Group, NS
Max	7.27 ± 0.09[†††]	7.25 ± 0.03[†††]	Interaction, NS	7.26 ± 0.06[†††]	7.26 ± 0.03[†††]	Interaction, NS
PaCO$_2$ (mm Hg)						
Rest	39.3 ± 2.3	39.2 ± 2.0	Exercise, $P < 0.0001$	36.2 ± 4.0	39.0 ± 2.7	Exercise, $P < 0.0001$
Max	31.1 ± 4.4[†††]	29.9 ± 2.4[†††]	Group, NS Interaction, NS	29.6 ± 3.0[†††]	31.7 ± 3.8[†††]	Group, $P < 0.01$ Interaction, NS
Erythrocyte 2,3-DPG (mmol·mL^{-1} red blood cells)						
Rest	3.32 ± 0.9	3.97 ± 0.5	Exercise, NS	3.50 ± 0.7	3.69 ± 0.9	Exercise, NS
Max	3.56 ± 1.0	3.97 ± 0.6	Group, NS Interaction, NS	3.64 ± 0.7	4.00 ± 0.9	Group, NS Interaction, NS
Metabolic data						
Plasma glucose (mM)						
Rest	5.2 ± 0.5	6.87 ± 1.9	Exercise, $P < 0.01$	5.3 ± 0.9	7.2 ± 3.2	Exercise, $P < 0.0001$
Max	6.8 ± 1.3[†]	7.52 ± 1.6	Group, $P = 0.052$ Interaction, NS	6.2 ± 0.8	8.8 ± 2.4[††*]	Group, $P < 0.01$ Interaction, NS

Values are means ± SD. "Rest" indicates values at rest just before the exercise, and "max" indicates values at exhaustion from the incremental exercise; NS, non significant.
The main effects from ANOVA are as follows: exercise, exercise effect, group, group effect, interaction, and exercise–group interaction.
Wilcoxon (variables only indicated at max):
[‡]Significantly different from their respective CON group, $P < 0.05$.
Mixed model *post hoc* ANOVA:
[*]Significantly different from their respective CON group, $P < 0.05$.
[†]Significantly different from rest, $P < 0.05$.
[††]Significantly different from rest, $P < 0.01$.
[†††]Significantly different from rest, $P < 0.001$.

applied 5 min before sampling) was collected to analyze lactate (amperometry, on ABL800 radiometer), factors able to modify the O$_2$Hb dissociation curve (pH, partial pressure of carbon dioxide (PaCO$_2$), by potentiometry, on ABL800 radiometer), and components of arterial O$_2$ content (CaO$_2$) (27) (arterial O$_2$ saturation (SaO$_2$) by spectrophotometry, partial pressure of O$_2$ (PaO$_2$) by amperometry, hemoglobin concentration by spectrophotometry, on ABL800 Radiometer). CaO$_2$ was calculated as the sum of bound (1.39 (hemoglobin) × SaO$_2$) and dissolved O$_2$ (0.003 PaO$_2$).

Statistical analyses. Statistics were computed using SAS 9.3 (SAS Institute, Inc., Cary, NC). Results are reported as means ± SD unless otherwise indicated. Normality was tested using the Shapiro–Wilk test. Demographic, anthropometric, and aerobic fitness data were compared between patients with type 1 diabetes and their respective group of healthy controls using the Wilcoxon matched-pairs test. NIRS data, arterialized O$_2$ transport, and blood factors able to influence the O$_2$Hb dissociation curve were compared between patients with type 1 diabetes and their respective control group using a linear mixed model

for repeated measurements. In this model, the fixed effects were the group effect (i.e., T1D-A vs CON-A and T1D-I vs CON-I), the exercise effect (repeated measurements corresponding to relative intensity levels—10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% of $\dot{V}O_{2max}$)—and the group–exercise interaction. The mixed model is an extension of the classical ANOVA allowing handling of correlations between repeated measurements. The choice of the covariance pattern model was based on the Akaike information criterion. The influence of each individual on the results was investigated using the Cook distance. If significant main effects or an interaction was observed with ANOVA, Bonferroni *post hoc* pairwise comparisons were applied. Pearson correlation coefficients were used to detect correlations between two continuous parametric variables. $P < 0.05$ was considered statistically significant.

RESULTS

Subjects' characteristics. Demographic and physical activity data from patients with type 1 diabetes and their matched healthy controls are summarized in Table 1. On the day of the exercise test, HbA_{1c} levels ranged between 8% and 10.3% in T1D-I and between 5.5% and 7.5% in T1D-A. The latter is explained by the fact that, for four of the 11 patients in the T1D-A group, HbA_{1c} changed from levels <7.0% the day of inclusion to levels between 7.0% and 7.5% on the day of exercise test. Plasma glucose concentrations
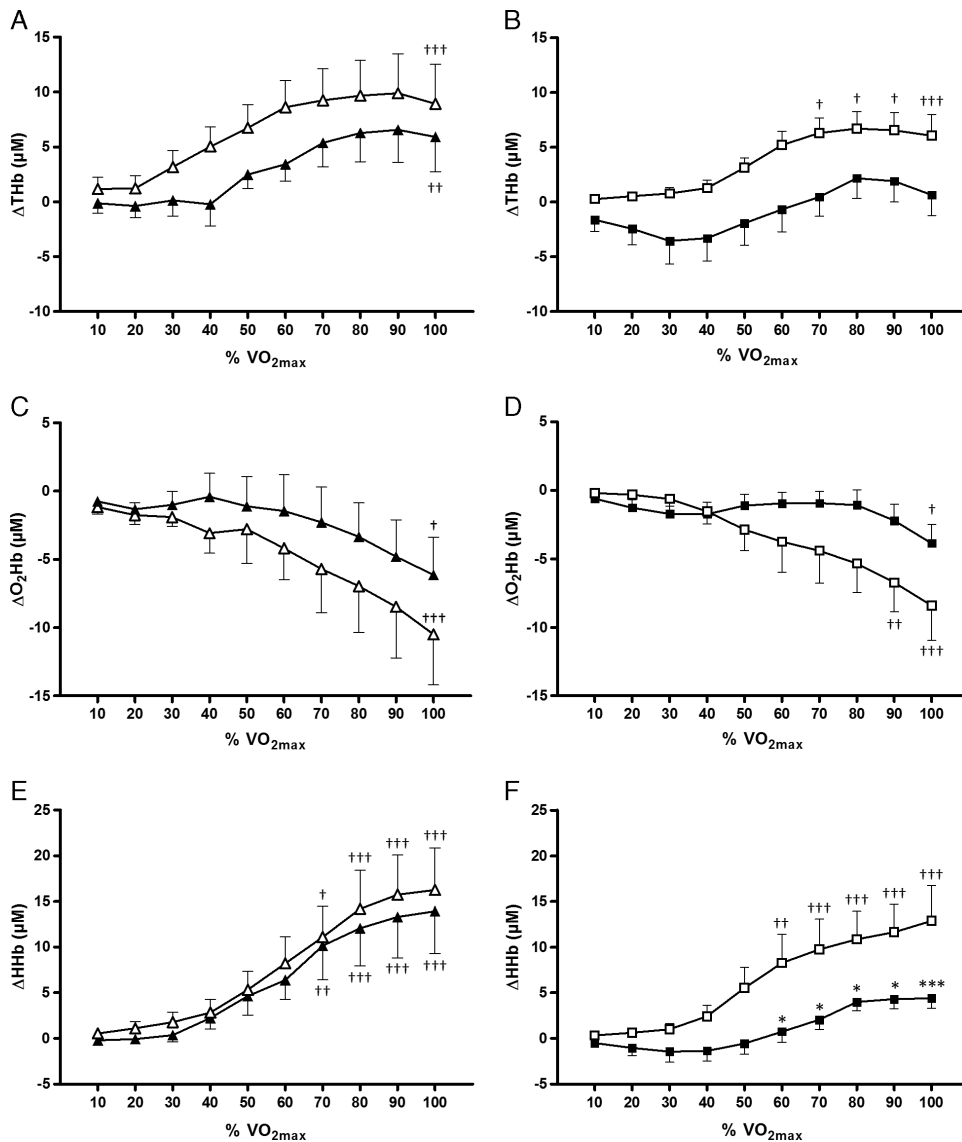
FIGURE 1—Recordings made by NIRS from the vastus lateralis. Change in ΔTHb (A and B), change in ΔO2Hb (C and D), and change in ΔHHb (E and F). Values are means ± SE. T1D-I, *black squares*; CON-I, *white squares*; T1D-A, *black triangles*; CON-A, *white triangles*. Post hoc analyses for group effect: significantly different from controls at *$P < 0.05$ and ***$P < 0.01$. Post hoc analyses for time effect: significantly different from rest at †$P < 0.05$, ††$P < 0.01$, and †††$P < 0.001$.

TABLE 3. Main effects by ANOVA regarding results from NIRS.

Variable	CON-A vs T1D-A		CON-I vs T1D-I	
	Main Effects by ANOVA (Relative Intensities)	Main Effects by ANOVA (Absolute Intensities)	Main Effects by ANOVA (Relative Intensities)	Main Effects by ANOVA (Absolute Intensities)
ΔTHb	Exercise, $P < 0.0001$	Exercise, $P < 0.0001$	Exercise, $P < 0.0001$	Exercise, $P < 0.0001$
	Group, NS	Group, NS	Group, $P < 0.01$	Group, $P < 0.01$
	Interaction, NS	Interaction, NS	Interaction, NS	Interaction, NS
$\Delta O_2 Hb$	Exercise, $P < 0.0001$	Exercise, $P < 0.05$	Exercise, $P < 0.0001$	Exercise, $P < 0.05$
	Group, NS	Group, NS	Group, NS	Group, NS
	Interaction, NS	Interaction, NS	Interaction, $P < 0.001$	Interaction, $P < 0.0001$
ΔHHb	Exercise, $P < 0.0001$	Exercise, $P < 0.0001$	Exercise, $P < 0.0001$	Exercise, $P < 0.0001$
	Group, NS	Group, NS	Group, $P < 0.0001$	Group, $P < 0.01$
	Interaction, NS	Interaction, NS	Interaction, $P < 0.01$	Interaction, $P < 0.01$

The main effects from ANOVA are as follows: exercise, exercise effect, group, group effect, interaction, and exercise–group interaction. Absolute intensities refer to exercise intensities expressed as absolute work rates, and relative intensities refer to exercise intensities expressed as relative $\dot{V}O_{2max}$.

increased during exercise in all groups (Table 2). None of the patients with type 1 diabetes became hypoglycemic during exercise.

$\dot{V}O_{2max}$. T1D-I had lower $\dot{V}O_{2max}$ than CON-I despite comparable levels of habitual physical activity (Table 1) as well as comparable HR achieved at exhaustion (Table 2). No significant difference in $\dot{V}O_{2max}$ was observed between T1D-A and CON-A (Table 2).

Arterial O_2 transport. CaO_2, and its components ([hemoglobin], SaO_2, PaO_2), did not differ significantly between T1D-I and CON-I during the exercise test. T1D-A had higher CaO_2 compared with CON-A, which could be explained by higher hemoglobin concentration. Levels and exercise-induced changes in PaO_2, SaO_2, and O_2 pulse were not significantly different between the groups (Table 2).

Muscle oxygenation and deoxygenation profiles. ΔTHb was significantly lower in T1D-I compared with that in CON-I during exercise, whereas no significant difference were observed between T1D-A and CON-A (Fig. 1 and Table 3).

$\Delta O_2 Hb$ decreased significantly less with exercise intensity in T1D-I compared with that in CON-I, whereas no intergroup differences were observed between T1D-A and CON-A (Fig. 1 and Table 3).

ΔHHb increased significantly in T1D-I, T1D-A, CON-I, and CON-A throughout the exercise test (Fig. 1 and Table 3). However, the levels of ΔHHb and the increase in ΔHHb were lower in T1D-I compared with those in CON-I (Table 3), particularly at exercise intensities above 60% of $\dot{V}O_{2max}$ (Fig. 1). In contrast, there were no differences in ΔHHb levels and changes between T1D-A and CON-A (Table 3).

Factors able to shift the $O_2 Hb$ dissociation curve. HbA_{1c} concentrations were significantly higher in T1D-I compared with those in both CON-I and T1D-A (Table 1).

There were no differences in erythrocyte 2,3-DPG levels and pH during exercise between patients with type 1 diabetes and their respective control groups (Table 2). $PaCO_2$ was higher in T1D-I compared with that in CON-I (Table 2).

In all the previous results sections, we found the same results when females were excluded from statistical analyses. In addition, the use of absolute work rates instead of relative intensity levels for the exercise effect in the mixed models did not change the results' sense (Table 3).

DISCUSSION

We found that patients with inadequate glycemic control but without any clinically detectable vascular complications displayed impaired aerobic capacity as well as reduction in blood volume and dramatic impairment in HHb increase in active skeletal muscles during intense exercise. However, regardless of their HbA_{1c} levels, patients with type 1 diabetes had adequate CaO_2. These results seem all the more relevant, given that this is the first *in vivo* study to assess all steps from O_2 delivery to release in the skeletal muscle during maximal exercise in patients with type 1 diabetes. In addition, the patients were divided into two groups having distinct levels of HbA_{1c} and all were free from clinical micro- and macroangiopathy. Another noteworthy feature of this study lies in the care taken to closely match each patient with a healthy control, taking into account the usual demographic data as well as the exact levels of physical activity (7-d accelerometry) (34). Regular physical activity is one of the major determinants of $\dot{V}O_{2max}$ (21,22) and thus could explain some discrepancies regarding aerobic fitness and type 1 diabetes reported in previous literature.

Aerobic fitness. Despite being relatively physically active (average of 41.3 ± 23.4 min of moderate to vigorous activities per day), the poorly controlled patients included in our study displayed a level of aerobic fitness ($\dot{V}O_{2max} = 34.6 \pm 7.2$ mL·kg^{-1}·min^{-1}) corresponding to levels usually observed in sedentary subjects (3), suggesting increased risk for cardiovascular diseases (2). In our study, the lower aerobic fitness in poorly controlled patients with type 1 diabetes compared with healthy controls is consistent with several studies in the literature (5,30), in which some patients undoubtedly suffered from micro- and macrovascular complications (30). Thus, besides the indirect effect of HbA_{1c} on aerobic fitness through the presence of chronic hyperglycemia-induced complications, our results raise the possibility of a direct effect of HbA_{1c} levels on $\dot{V}O_{2max}$. The mechanisms underlying

this direct relation may involve muscle O_2 delivery (including arterial O_2 transport and muscle blood perfusion) and/or O_2 release to muscles, which are two main determinants of $\dot{V}O_{2max}$ (6,37).

Arterial O_2 transport. Arterial O_2 transport is dependent on two key factors. The first is the ability of the lungs to oxygenate the blood as it passes through the pulmonary capillary network. In the current study, this was reflected by the O_2 content of arterialized blood (27). O_2 transport also depends on cardiac output, which is determined by the product of stroke volume (as reflected by O_2 pulse) and HR. We observed that arterial O_2 transport capacity was comparable in poorly controlled patients with type 1 diabetes and their healthy controls. This finding suggests that, in patients with type 1 diabetes but free from clinically detectable microangiopathy, the increase in pulmonary capillary blood flow and alveolar–capillary diffusion induced by high-intensity exercise does not highlight any limitation in lung function. In agreement with our results, Wanke et al. (38) showed that patients with type 1 diabetes, in the absence of overt pulmonary disease, have a normal alveolar–arterial O_2 gradient at comparable power outputs.

Blood volume in active skeletal muscles. Changes in THb are thought to reflect changes in tissue blood volume (19). Therefore, the significant increase in ΔTHb in both groups of healthy controls and of patients with adequate glycemic control in the present study is consistent with the increase in muscle blood volume usually observed with increasing exercise intensity (7). However, in cases of inadequate glycemic control, patients with type 1 diabetes had significantly lower ΔTHb than their healthy controls. Our results supplement those of Pichler et al. (33). In children and adolescents with type 1 diabetes, among whom some were poorly controlled (mean HbA_{1c}, 9.2% ± 1.8%), the authors found lower ΔTHb at the forearm muscle in response to a short submaximal local exercise (1-min rhythmic handgrip) performed in addition to provoked nonphysiological increase in forearm arterial inflow. The latter was artificially set up using brachial venous occlusion (three occlusions of 20 s interspaced by a rest period of 40 s). The current study suggests that, even without a preconditioning stress such as venous occlusion, the physiological condition of maximal exercise was sufficient to induce alteration in muscle perfusion. This alteration is possibly favored by endothelial dysfunction and/or functional alterations of the microcirculation occurring even before overt microvascular complications in cases of chronic hyperglycemia (i.e., high HbA_{1c} levels) and/or in cases of long-term diabetes. The poorly controlled patients, indeed, had longer duration of disease than the well-controlled patients in our study.

The exercise-induced HHb increase in active muscles. There were no differences in ΔHHb levels and its increase between patients with adequate glycemic control and their healthy controls. These results coincide with those of Peltonen et al. (32), who reported a comparable level of ΔHHb at maximal exercise in patients with type 1 diabetes

with an HbA_{1c} of 7.7% ± 0.7% and healthy subjects. We did, however, find that patients with inadequate glycemic control displayed dramatic impairment in exercise-induced ΔHHb increase and reduced ΔHHb levels, especially with intense exercise (>60% $\dot{V}O_{2max}$).

During a bout of exercise, several factors may explain the reduction in ΔHHb increase. First, a lower ΔHHb may be observed in the case of better matching between muscle O_2 delivery (particularly depending on CaO_2 and muscle blood perfusion) and muscle O_2 use. For example, this scenario is seen at the onset of heavy-intensity exercise in young healthy adults compared with older healthy adults because the former displays better increase in muscle blood volume (as reflected by ΔTHb) for a comparable need for O_2 for muscle contraction (13). However, this mechanism does not explain the ΔHHb impairment in our poorly controlled patients with type 1 diabetes, as they otherwise displayed lower ΔTHb rates during the exercise test.

Secondly, the lower ΔHHb may be explained by lower muscle O_2 extraction (19). This could occur in two situations, as follows: 1) reduced capacity of O_2Hb dissociation, which can occur in pathological circumstances (e.g., CO intoxication) or in sport (e.g., hyperventilation-induced alkalosis in mountaineering), and 2) reduced tissue capacity of O_2 use (e.g., mitochondrial dysfunction). The hypothesis of the former situation (i.e., a disturbed O_2Hb dissociation rate) is probably involved in the lower exercise level of ΔHHb found *in vivo* in our poorly controlled (HbA_{1c}, >8%) patients. Indeed, it has been shown *in vitro* that glycation of hemoglobin, at percentages that might be found in patients with diabetes (i.e., 8% HbA_{1c}), reduced the kinetics of hemoglobin O_2 release by 10% in comparison with a 4% HbA_{1c} level (25). In our study, the possibility of higher O_2 affinity of HbA_{1c} seems all the more likely, with the consideration that other stimuli, able to shift the O_2Hb dissociation curve to the right during intense exercise (Bohr effect) (26), were either comparable (blood pH, erythrocyte 2,3-DPG) or higher ($PaCO_2$) in the poorly controlled patients compared with those in healthy controls.

Thirdly, we cannot exclude the possibility that the exercise-induced switch of muscle blood flow from the "nonnutritive" route (i.e., the flow "reserve" irrigating muscle connective tissues and their associated adipocytes) to the "nutritive" route (i.e., capillaries in intimate contact with the skeletal muscle fibrils) (12) was impaired in our poorly controlled patients with long-standing diabetes, hence reducing overall O_2 extraction proportion. This phenomenon has never been investigated *in vivo* during exercise in humans with type 1 diabetes but can be suggested through previous work on animal models of diabetes (31).

Notwithstanding, the lower HHb increase in the vastus lateralis muscle found in the patients with inadequate glycemic control may contribute to their impaired aerobic fitness because we detected significant positive correlation between $\dot{V}O_{2max}$ and ΔHHb at maximal exercise in all the patients with type 1 diabetes ($r = 0.54$, $P < 0.01$).

Further studies are needed to determine whether the blunted HHb increase in muscles observed in patients with poor glycemic control may also be related to impaired muscle mitochondrial function.

CONCLUSIONS

In summary, the ΔHHb increase in the vastus lateralis during maximal exercise is blunted in patients with type 1 diabetes and with high levels of HbA_{1c}. This result, obtained *in vivo* during a physiological condition, supports the hypotheses of an increase in O_2 affinity induced by hemoglobin glycation and/or of a disturbed balance between nutritive and nonnutritive muscle blood flow routes, as previously put forward by *in vitro* and *animal* studies, respectively. This finding is of particular clinical relevance, considering the negative correlation between ΔHHb increase and $\dot{V}O_{2max}$ found in patients with type 1 diabetes in the current study.

Ultimately, from a practical perspective, maximal exercise coupled with NIRS measurement represents a promising noninvasive method of physiologically assessing disorders of muscle perfusion in patients without otherwise clinically detectable microangiopathy. Determining whether these disorders of muscle blood expansion can be reversed with HbA_{1c}

improvement will be a challenge for future prospective clinical trials.

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