Supplemental data

Beneficial effects of endurance exercise training on skeletal muscle microvasculature in sickle cell disease patients by Merlet al.

Methods

Exclusion criteria

Exclusion criteria were: participation in another clinical trial, chronic transfusions, acute transfusion in the prior 3 months, chronic inflammatory or infectious disease, any intercurrent medical event within the month preceding inclusion, clinical signs and/or history of heart failure, left ventricular ejection fraction < 50%, pulmonary arterial hypertension with tricuspid regurgitation velocity > 2.8 m/s, atrial fibrillation, ventricular arrhythmias, left ventricular hypertrophy, significant valvulopathy, known coronary disease, uncontrolled hypertension, treatment with anti-arrhythmia drugs including β-blockers, current anti-coagulant treatment, pacemaker or defibrillator, known cerebral vasculopathy, prior stroke, epilepsy, pregnancy or lactation, hip osteonecrosis and/or the incapacity to understand the aims, design and possible risks of the study protocol

Submaximal cardiopulmonary exercise tests

Exercises were performed on an Ergoselect Confort cycle ergometer (General Electric, USA). $\dot{V}O_2$ and $[lactate]_b$, were measured by ErgoCard (Medisoft, Sorinnes, Belgium) and Lactate Scout+ (EKF diagnostics, Cardiff, UK) devices, respectively. Muscle oxygenation

was investigated with a portable near-infrared spectroscopy apparatus (Portalite, Artinis Medical Systems, the Netherlands). The probe was affixed to the skin of the lower third of the right *vastus lateralis*, parallel to muscle fibers, in a position normalized to ensure accurate post-intervention repositioning. The device was a 2-wavelength continuous wave system, which simultaneously uses the modified Beer-Lambert law and spatially resolved spectroscopy method to measure oxy- and deoxy-hemoglobin changes from the differences in absorption characteristics of the light (750 and 850 nm) and to compute a tissue saturation index (TSI, %), reflecting the dynamic balance between O_2 supply and O_2 consumption in the investigated muscle volume.[1] Rating of perceived exertion (RPE) was assessed using the CR10 Scale® (category scale labelled 0-10).[2] The exercise was also used to determine the first lactate threshold (LT1) which corresponds to the first inflection point in the $[lactate]_b$ vs. power output curve.[2]

Endurance exercise training protocol

Training workload had to be low enough to avoid acidosis and therefore to be safe for the patients, but also high enough to induce muscle adaptations. In that sense, a training workload eliciting a target value of 2.5 mmol/L of blood lactate concentration was chosen.[2] For the training session, the workload of session N was adapted according to the blood lactate concentration measured during the training session N-1. The change in workload followed the strategy: 2.5±0.3 mmol/L = no change, 2.9–3.2 mmol/L = -5%, 3.3–3.6 mmol/L = -10%, >3.7 mmol/L = -20%, 1.9–2.1 mmol/L = +5%, 1.6–1.8 mmol/L = +10%, <1.5 mmol/L = +20%.[2]

Results and Discussion

Effects of hydroxyurea (HU).

Due to the clinical and biological benefits of HU treatment in SCD (dependent or independent of fetal Hb), the hypothesis that HU could influence (1) muscle characteristics at baseline and (2) benefits (muscular and functional) in response to endurance-training is justified. In the present study, 15 patients were receiving HU. Ten patients were at a stable dose for more than a year and three others were at a stable dose for more than 6 months. In one patient, HU was initiated less than 6 months prior to enrollment. Most importantly, in none of the patients was treatment with HU initiated just before enrollment or during the protocol. Among the15 patients under HU, 6 [4 women and 2 men] were in the non-training group and 9 [2 women and 7 men] were in the training group. Baseline measures of patients receiving HU (n = 15) or not receiving HU (n = 17) were similar (Supplemental Table 1). Thus, it appears that HU has no or undetectable effects on muscle microvascular characteristics. However, since HU is administered in the most fragile patients, with hypothetically very poor muscle microvascular characteristics,[3] one might conclude that HU ameliorates the microvasculature of the more severely affected patients to match that of the less severely affected patients. The present study does not allow definitive conclusions to be made on this point meaning further studies are necessary. In the training group, nine of 15 patients were receiving HU (Supplemental Table 2). Overall, it appears that in the training group HU did not seem to enhance observed adaptations. Indeed, among the 17 parameters investigated, only two may have resulted in greater adaptations in the

training patients with HU (LC and TSI). From this analysis, the hypothesis that HU could enhance the benefits (muscular and functional) in the context of exercise training cannot be validated. However, this is a very interesting point that deserves to be more specifically investigated.

Responders vs. non-responders.

Considering the training group and the parameters that were significantly improved by endurance training [CD, CAF all fibers (mean), CAF type I fibers, LC, LC/PF all fibers (mean), LC/PF type I fibers and LC/PF type IIa fibers], no correlations between the initial values and the changes in response to endurance training were obtained. Furthermore, if one considers as non-responders the patients with a parameter which changes in the opposite way as expected, all training patients (15) were responders for LC/PF all fibers (mean) and LC/PF type I fibers. One can also observe (1) one non-responder for CAF type I fibers and for LC, (2) two non-responders for CAF all fibers (mean) and LC/PF type IIa fibers, (3) four non-responders for CD. If one extends the analysis to the integrative parameters, all patients were responders for both power output and $\dot{V}O_2$ at LT1, while there were four non-responders for blood lactate concentrations at 40/60 W. The number of patients is small, and if one takes into account the lack of correlations and the disparity in response among parameters, it is difficult to state if some training patients can be defined as non-responders.

Supplementary references

1. Ferrari M, Muthalib M, Quaresima V. The use of near-infrared spectroscopy in understanding skeletal muscle physiology: recent developments. *Philos Trans A Math Phys Eng Sci.* 2011;369(1955):4577-90.
2. Borg G. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc*. 1982;14(5):377–81.
3. Messonnier LA, Gellen B, Lacroix R, et al. Physiological Evaluation for Endurance Exercise Prescription in Sickle Cell Disease. *Med Sci Sports Exerc.* 2019.
4. Ravelojaona M, Féasson L, Oyono-Enguéllé S, et al. Evidence for a profound remodeling of skeletal muscle and its microvasculature in sickle cell anemia. *Am J Pathol.* 2015;185(5):1448-56.

Supplemental Table 1. Baseline microvascular characteristics and integrative data by hydroxyurea (HU) treatment status in SCD patients

Variables	Patients not under HU n = 17	Patients under HU n = 15	*P*
Microvascular characteristics			
Morphological characteristics			
CP, µm	36.8 (9.3)	37.4 (10.4)	.858
CSA, µm²	65.6 [38.0; 78.1]	63.2 [40.1; 72.1]	.985
COD, µm	6.0 [4.8; 6.7]	6.2 [4.8; 6.6]	.925
COD < 5 µm, %	31.0 [16.0; 64.4]	22.4 [16.0; 59.5]	.208
5 < COD < 10 µm, %	65.5 [33.3; 78.2]	56.5 [39.6; 74.8]	.264
COD > 10 µm, %	1.3 [0.0; 3.2]	1.9 [0.0; 6.9]	.439
CapTor, A.U.	2.15 (0.12)	2.13 (0.12)	.593
Quantitative characteristics			
CD, cap/mm²	191 (30)	201 (33)	.392
CAF, cap/fiber			
All fibers (mean)	2.3 [2.0; 2.6]	2.5 [2.2; 2. 8]	.299
Type I fibers	2.7 [2.4; 3.1]	2.9 [2.6; 3.4]	.396
Type IIa fibers	2.2 [1.6; 2.4]	2.1 [2.0; 2.5]	.406
Type IIx fibers	1.4 [1.1; 1.5]	1.5 [1.2; 1.7]	.483
Functional indexes			
LC, µm	26.0 (10.6)	29.6 (10.8)	.352
LC/PF, %			
All fibers (mean)	10.6 (3.1)	12.0 (3.4)	.223
Type I fibers	12.6 (3.1)	14.3 (3.2)	.140
Type IIa fibers	9.7 (3.3)	11.0 (2.3)	.255
Type IIx fibers	5.9 [4.9; 8.2]	7.1 [4.3; 8.1]	.901
CD*CSA, µm²/mm²	13,583 (6,107)	15,326 (7,263)	.467
CD*CSA*Hb, A.U.	1.26 (0.61)	1.43 (0.94)	.550
Integrative data			
At the first lactate threshold			
Power output, W	30.0 [+30.0; +40.0]	40.0 [+30.0; +45.0]	.212
$\dot{V}O_2$, L/min	0.60 [0.54; 0.80]	0.66 [0.61; 0.80]	.233
TSI, %	65.3 [61.4; 66.7]	67.3 [64.3; 68.3]	.129
At 40 W (women) or 60 W (men)			
$[lactate]_b$, mmol/L	2.7 (0.8)	2.7 (0.7)	.888
RPE, A.U.	2.0 [2.2; 5.5]	2.0 [1.0; 4.8]	.622

Results are expressed as mean (SD) or median [IQR]. A.U., arbitrary units.

Supplemental Table 2. Effect of the training program on microvascular characteristics and integrative data by hydroxyurea (HU) treatment status in training SCD patients

Variables	Training patients not under HU n = 6 Relative change (%)	Training patients under HU n = 9 Relative change (%)	*P*
Microvascular characteristics			
Morphological characteristics			
CP, µm	+8.2% (30.1)	+25.4% (25.3)	.253
CSA, µm²	+13.3% (47.7)	+55.9% (51.4)	.130
COD, µm	+3.5% (21.4)	+22.2% (26.1)	.168
COD < 5 µm, %	+42.2% (165)	-17.0% (96.3)	.195
5 < COD < 10 µm, %	+12.1% (36.1)	+82.0% (94.6)	.239
CapTor, A.U.	+2.6% (8.49)	+1.7% (9.9)	.859
Quantitative characteristics			
CD, cap/mm²	+13.1% (21.2)	+20.1% (12.4)	.432
CAF, cap/fiber			
All fibers (mean)	+11.6% (17.3)	+22.5% (19.1)	.282
Type I fibers	+20.9% (19.3)	+24.3% (15.2)	.710
Type IIa fibers	+12.8% (25.2)	+13.7% (25.3)	.945
Type IIx fibers	+35.1% (32.7)	+6.6% (37.4)	.294
Functional indexes			
LC, µm	+27.2% (22.5)	+62.6% (35.1)	**.049**
LC/PF, %			
All fibers (mean)	+27.0% (20.8)	+52.7% (36.3)	.142
Type I fibers	+33.4% (19.4)	+57.1% (37.1)	.176
Type IIa fibers	+35.9% (34.1)	+34.7% (35.6)	.949
Type IIx fibers	+25.8% [+12.0; +29.6]	+15.4% [+3.6; +26.0]	.327
CD*CSA, µm²/mm²	+22.2% (28.3)	+85.3% (74.3)	.071
CD*CSA*Hb, A.U.	+18.6% (20.7)	+88.3% (95.6	.126
Integrative data			
At the first lactate threshold			
Power output, W	+40.0% (20.7)	+47.6% (20.4)	.493
$\dot{V}O_2$, L/min	+19.3% [9.9; +27.6]	+26.1% [+18.9; +59.2]	.157
TSI, %	-161% [-240; -78.9]	-14.6% [-41.8 ; +14.8]	**.025**
At 40 W (women) or 60 W (men)			
$[lactate]_b$, mmol/L	-12.0% (24.3)	-22.7% (16.3)	.324
RPE, A.U.	-72.2% (21.0)	-81.3% (9.8)	.361

Results are expressed as mean (SD) or median [IQR]. A.U., arbitrary units. **Bold** results are statistically significant differences at $P < .05$.

Supplemental Table 3. Effect of the training program on microvascular characteristics and integrative data to exercise by sex in training SCD patients

Variables	Training men n = 8 Relative change (%)	Training women n = 7 Relative change (%)	*P*
Microvascular characteristics			
Morphological characteristics			
CP, µm	+14.5% (31.8)	+23.1% (23.7)	.565
CSA, µm²	+36.2% (61.1)	+41.9% (46.2)	.845
COD, µm	+10.0% (27.8)	+20.1% (23.1)	.464
COD < 5 µm, %	-14.1% [-66.9; +105.0]	-50.9% [-65.3; -11.8]	.418
5 < COD < 10 µm, %	+9.7% [-18.1; +123.0]	+26.8% [+11.9; +91.1]	.487
COD > 10 µm, %	-10.5% (31.6)	+107.0% (106.0)	.084
CapTor, A.U.	+5.8% (8.9)	-2.2% (7.7)	.088
Quantitative characteristics			
CD, cap/mm²	+21.8% (13.7)	+12.1% (18.2)	.261
CAF, cap/fiber			
All fibers (mean)	+18.6% (18.9)	+17.5% (19.7)	.911
Type I fibers	+21.7% (14.9)	+24.4% (19.1)	.757
Type IIa fibers	+11.4% (26.4)	+15.5% (23.7)	.761
Type IIx fibers	-0.9% (41.9)	+34.0% (28.4)	.203
Functional indexes			
LC, µm	+47.5% (34.7)	+49.6% (37.5)	.913
LC/PF, %			
All fibers (mean)	+41.5% (38.7)	+43.5% (27.6)	.913
Type I fibers	+42.4% (35.5)	+53.6% (30.6)	.525
Type IIa fibers	+30.6% (40.3)	+40.4% (26.4)	.591
Type IIx fibers	+10.1% [+3.6; +12.9]	+29.6% [+25.8; +41.4]	.054
CD*CSA, µm²/mm²	+65.8% (82.2)	+53.6% (49.4)	.739
CD*CSA*Hb, A.U.	+30.8% [-0.14; +130.0]	+28.0% [+13.9; +72.9]	1.000
Integrative data			
At the first lactate threshold			
Power output, W	+45.2% (24.0)	+43.8% (16.5)	.897
$\dot{V}O_2$, L/min	+35.5% [+16.0; +59.3]	+19.3% [+16.4; +26.6]	.355
TSI, %	-35.0% [-68.4; -7.3]	-68.4% [-230.0; +0.8]	.563
At 40 W (women) or 60 W (men)			
$[lactate]_b$, mmol/L	-18.3% (20.5)	-18.5% (20.6)	.987
RPE, A.U.	-87.3% (11.0)	-82.0% (18.8)	.533

Results are expressed as mean (SD) or median [IQR]. A.U., arbitrary units.

Supplemental Table 4. Changes on microvascular characteristics and integrative data by hospitalization status in non-training SCD patients

Variables	Non-training hospitalized patients n = 4 Relative change (%)	Non-training non-hospitalized patients n = 13 Relative change (%)	*P*
Microvascular characteristics			
Morphological characteristics			
CP, µm	+0.2% [-8.9; +11.2]	-1.0% [-18.3; 21.7]	.821
CSA, µm²	+3.0% [-2.8; +10.3]	-3.1% [-22.8; +21.4]	.821
COD, µm	-0.5% [-1.71; +0.48]	-1.9% [-9.9; +12.7]	.910
COD < 5 µm, %	-6.3% [-23.8; +7.9]	-0.9% [-53.1; +69.5]	.903
5 < COD < 10 µm, %	+4.3% [-10.5; +22.9]	+3.1% [-4.87; +42.9]	.651
COD > 10 µm, %	+48.6% [-6.6; +104.0]	-14.3% [-59.4; +38.4]	1.00
CapTor, A.U.	+5.4% [+0.2; +11.6]	-0.7% [-3.58; +3.51]	.213
Quantitative characteristics			
CD, cap/mm²	+12.7% (16.5)	+12.4% (53.3)	.258
CAF, cap/fiber			
All fibers (mean)	+11.7% (7.9)	-5.9% (26.1)	.211
Type I fibers	+3.9% (12.8)	-7.4% (23.7)	.381
Type IIa fibers	+21.1% (12.2)	-2.5% (21.5)	.056
Type IIx fibers	+22.7% [+0.6; +89.6]	-3.2% [-20.4; +6.3]	.310
Functional indexes			
LC, µm	+5.8% [-1.4; +9.1]	-20.0% [-29.1; +0.5]	.141
LC/PF, %			
All fibers (mean)	+7.0% (7.1)	-11.1% (24.7)	.178
Type I fibers	+5.7% (26.2)	-12.1% (21.4)	.188
Type IIa fibers	-2.9% (11.6)	-10.2% (23.7)	.572
Type IIx fibers	+99.6% (129.0)	+17.9% (65.8)	.154
CD*CSA, µm²/mm²	+22.0% [+11.6; +27.1]	-0.5% [-16.0; +16.0]	.308
CD*CSA*Hb, A.U.	+7.4% (8.3)	+1.1% (8.7)	.221
Integrative data			
At the first lactate threshold			
Power output, W	+18.8% (42.7)	-1.3% (24.8)	.249
$\dot{V}O_2$, L/min	-3.9% (13.3)	+0.4% (14.6)	.604
TSI, %	-90.0% [-121.0; -85.1]	-68.9% [-213.0; -6.9]	.365
At 40 W (women) or 60 W (men)			
$[lactate]_b$, mmol/L	-2.4% (17.2)	+7.6% (27.9)	.516
RPE, A.U.	0.0% [-16.7; +0.0]	-33.3% [-66.7; +50.0]	.853

Results are expressed as mean (SD) or median [IQR]. A.U., arbitrary units.

Appendices

Written on behalf of the EXDRE collaborative study group.

EXDRE collaborative study group

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