HIGH-RESOLUTION RESPIROMETRY AS A TOOL TO ASSESS SKELETAL MUSCLE MITOCHONDRIAL STATE IN PATIENTS WITH HUNTINGTON'S DISEASE

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Experiment design

Table 1. Summary of clinical details

Group	Subgroup	Number of people	Sex	Mean age	CAG1	CAG2	UHDRS (MA)	BMI
Control	PM Control	4	♂	28.5±3.21	-	-	-	24.28±1.47
		4	♀					
	M Control	3	♂	40.29±4.82	-	-	-	25.86±1.24
		4	♀					
HD	Premanifest (PM)	4	♂	27±3.25	<23.88±6.96	47.75±5.23	1±0.93	23.68±1.79
		4	♀					
	Manifest (M)	4	♂	43.13±5.59	20.88±6.03	46±2.62	42.38±10.07	22.34±2.36
		4	♀					

Data are means±SD

Fine-needle biopsy of the m. rectus femoris

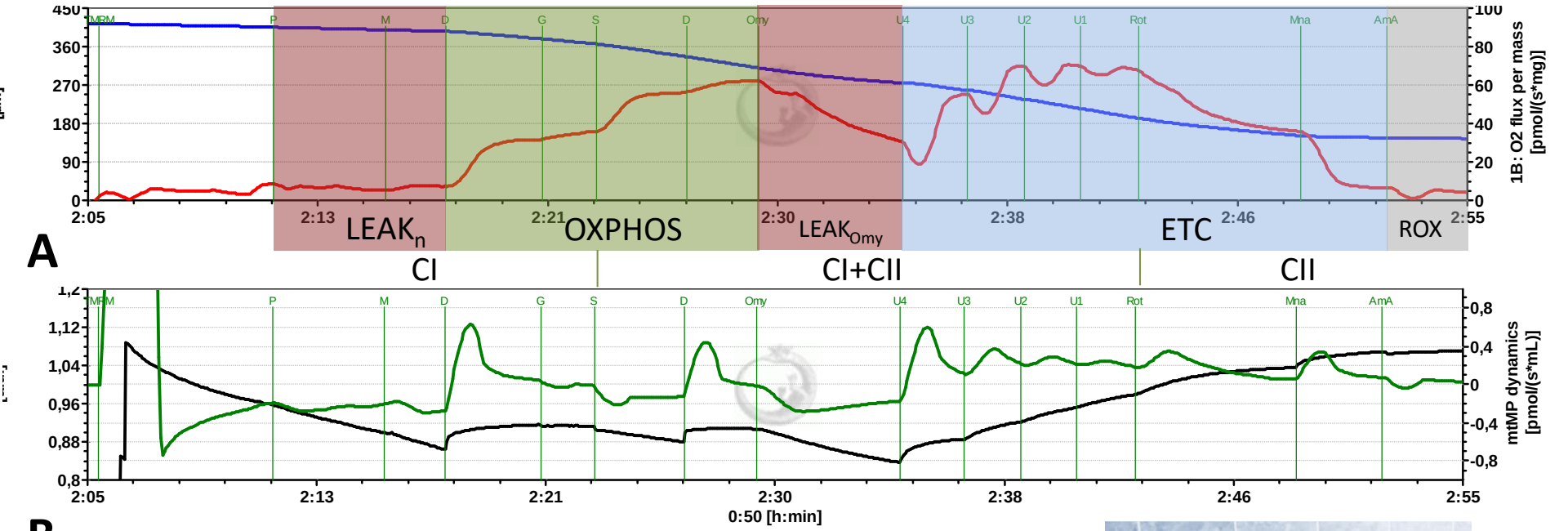
Mechanical preparation of muscle tissue*

Permeabilization in 50 µg/mL saponin solution*

High-Resolution Respirometry, mtMP (HRR, Oroboros Oxygraph-2k, Austria)

* - according to Doerrier et al (2018) High-Resolution FluoRespirometry and OXPHOS Protocols for Human Cells, Permeabilized Fibers from Small Biopsies of Muscle, and Isolated Mitochondria // Mitochondrial Bioenergetics. Methods in Molecular Biology, vol 1782.

Method: HRR



B

LEAK - uncoupled respiration during the non-phosphorylating resting state;

OXPHOS - coupled respiration during oxidative phosphorylation;

ETC - electron transfer system capacity during noncoupled respiration;

ROX - residual oxygen consumption;

mtMP – mitochondrial membrane potential

C

Fig.1. A – graph of skeletal muscle respiration (oxygen concentration, blue line; respiration rate, red line), B – graph of mtMP (mtMP, black line; mtMP dynamics, green line), C – Oroboros O2k-Respirometer (Oroboros Instruments, Austria)

Results

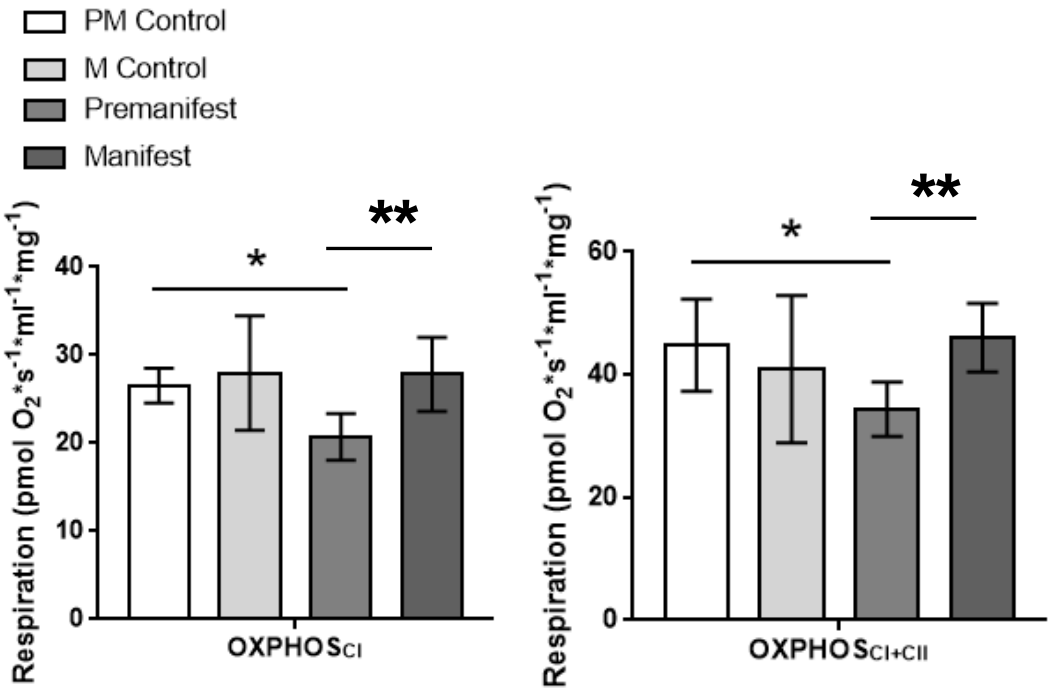


Fig. 3. *Coupled* respiration during oxidative phosphorylation (OXPHOS) is decreased in premanifest HD patients both for complex I (CI) and complexes I+II (CI+CII)

		$OXPHOS_{CI}$ (pmol O_2 s^{-1} ml^{-1} mg^{-1})	$OXPHOS_{CII}$ (pmol O_2 s^{-1} ml^{-1} mg^{-1})	$OXPHOS_{CI+CII}$ (pmol O_2 s^{-1} ml^{-1} mg^{-1})
Control	**PM Control**	26,5541 ± 1,9626	18,2448 ± 7,4554	44,7989 ± 7,4959
	M Control	27,9434 ± 6,4966	12,9254 ± 5,9286	40,8687 ± 12,0068
HD	**Premanifest (PM)**	20,7371 ± 2,6575*	13,6076 ± 5,2996	34,3447 ± 4,4222*
	Manifest (M)	27,8112 ± 4,1924**	18,2433 ± 5,9322	46,0546 ± 5,5781**

Data are means±SD, *n*=7-8. * = significant difference with matched control, ** = significant difference with premanifest

Results

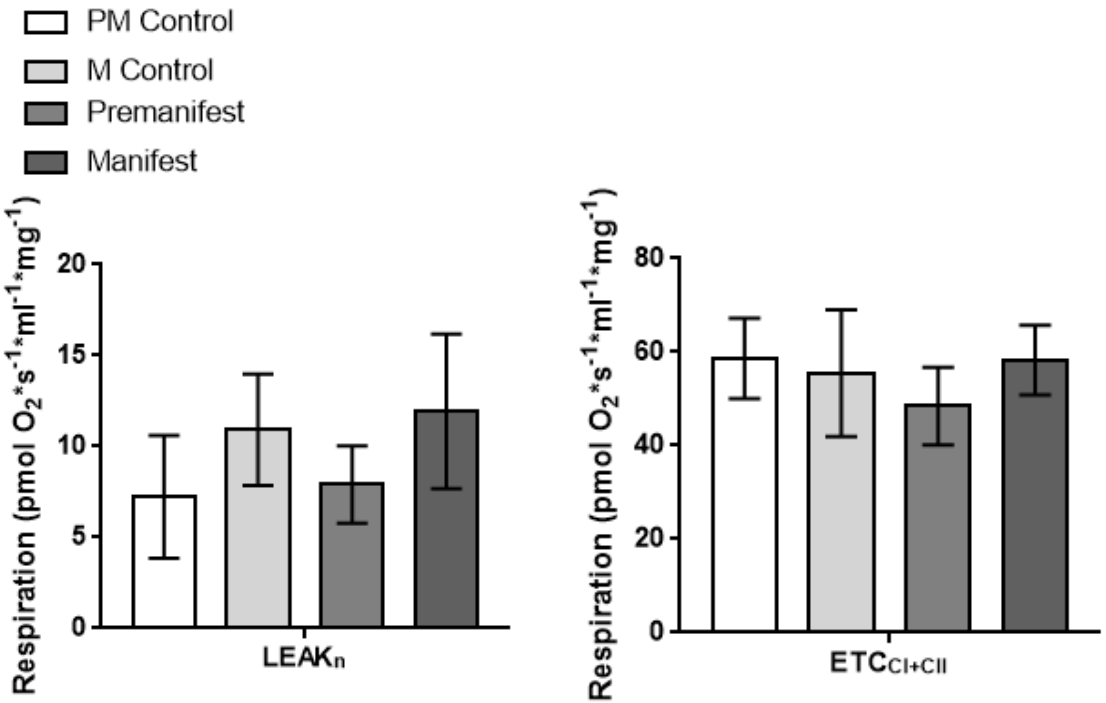


Fig. 4. No significant differences between controls and HD patients were observed in *uncoupled* respiration during the non-phosphorylating resting state ($LEAK_n$), and in *noncoupled* respiration (ETC_{CI+CII})

		$LEAK_n$ (pmol O_2 s^{-1} ml^{-1} mg^{-1})	ETC_{CI+CII} (pmol O_2 s^{-1} ml^{-1} mg^{-1})	ET-C_{CII} (pmol O_2 s^{-1} ml^{-1} mg^{-1})
Control	**PM Control**	7,2270 ± 3,3743	58,6285 ± 8,6164	27,3540 ± 6,1773
	M Control	10,9205 ± 3,0547	55,4783 ± 13,5851	23,0443 ± 4,5433
HD	**Premanifest (PM)**	7,9054 ± 2,1263	48,4246 ± 8,2777	23,6001 ± 4,4871
	Manifest (M)	11,9351 ± 4,2412	58,3268 ± 7,4686	29,9741 ± 6,7018

Data are means±SD, *n*=7-8

Conclusion

HRR can be used as a promising tool to assess mitochondrial state in skeletal muscles from patients with HD.

Our results demonstrate alterations in coupled mitochondrial respiration of skeletal muscle observed in the premanifest stage of Huntington's Disease (HD). Analyzing male and female cohorts separately, it was shown that the observed decrease in respiration rates of premanifest patients is sex-independent.

The dynamics of mtMP was on the same level in HD patients and matched controls. The tendency to less pronounced changes in mtMP were observed in premanifest and manifest patients in comparison to matched controls.

According to the data, the function of the mitochondrial respiratory chain and ATP production can be limiting factors for the aerobic metabolism of skeletal muscles in the early stages of HD.