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Systemic response to acute aerobic exercise in the circulatory system: a possible cross-talk between plasma extracellular vesicles and blood monocytes

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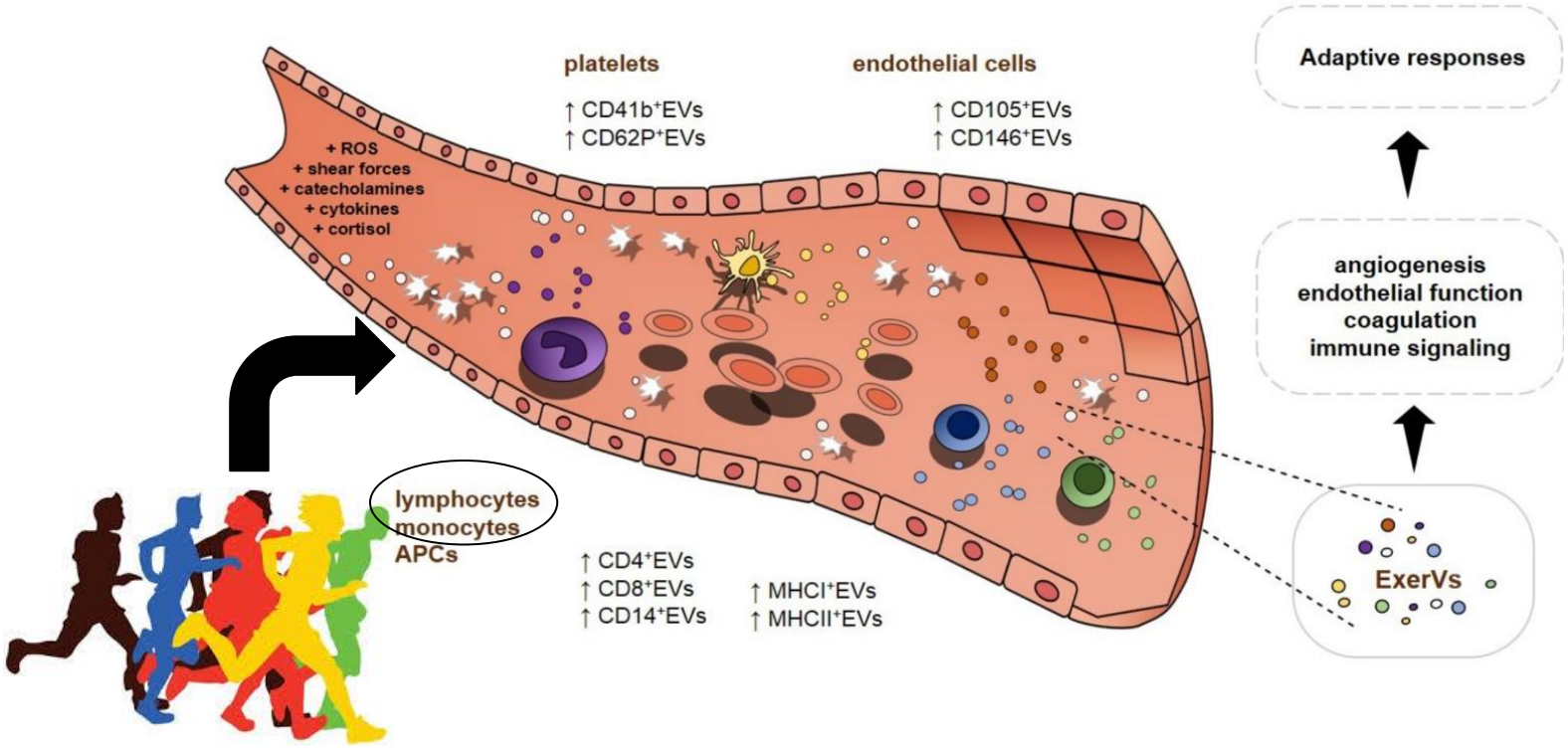
Abstract

Extracellular vesicles (EVs) mediate cell-cell communication in different physiological conditions, as well as during physical exercise (PE). Since peripheral blood mononuclear cells (PBMCs) can release and internalize plasma-EVs, the aim of this work was to investigate the relationship between the modulation exerted by fitness levels and acute aerobic exercise (EX) - 30'on treadmill, 70% HRmax - on EV-cargo and PBMC protein expression. Analysis focused on molecules involved in the stress response, such as antioxidant enzymes and heat shock proteins, as well as markers of oxidative stress [lipid peroxidation (LP) and protein carbonylation (PCO)]. To this aim, two groups of healthy young males, categorized by different VO_{2max} values (Untrained, UTS, 7: 41.8 ± 3.8 and Trained, TS, 7: 48.5 ± 3.2), were included in the study. Plasma-EVs and PBMCs were isolated from blood samples collected at baseline, 3hrs and 24hrs after EX. Our data demonstrated that UTS-EVs had higher PCO and LP levels than TS-EVs while in PBMC this difference is only seen for PCO ($p<0,05$), with no modulation by EX. Plasma-EVs shuttle antioxidant enzymes (SOD1, SOD2, Catalase and TrxR1) and HSP70, present in PBMCs. EVs and PBMCs basal levels of SOD1 and TrxR1 were not different between groups, with an EX-related increase of SOD1 exclusively in PBMCs of UTS 24hrs post-EX ($p<0.05$). SOD2 basal levels were significantly higher in PBMCs and EVs from UTS compared to TS ($p<0.05$), while Catalase was significantly higher in UTS-EVs ($p<0.05$), but not in UTS-PMBCs. No modulation of SOD2 or Catalase was exerted by EX. HSP70 appears modulated by fitness levels and EX only in PBMCs, with a higher basal expression in TS than in UTS and a significant increase in UTS at 24hrs post-EX. These data suggest a link between PBMCs and plasma-EVs, with a possible communication following the exercise-induced modifications concerning redox homeostasis.

Introduction

Acute exercise (EX) is a physiological stressor that alters cellular homeostasis and triggers the release of a plethora of molecules (proteins, lipids, and nucleic acids) carried by the Extracellular vesicles (EVs). Blood cells such as lymphocytes (PBMCs) can release and internalize EVs, possibly contributing to the adaptive systemic signaling mechanism associated with physical exercise.



Alexandra Brahmer, 2021

Aim

Given the role of acute exercise to perturb redox homeostasis in different kinds of cells, including PBMCs, and the evidence which describes the functional molecules transported by EVs between cells, we aimed to verify the effect of a single bout of acute exercise (EX) in trained (TS) and untrained (UTS) healthy young males. Main outcomes were:

- ✓ Concentration and size distribution of EV released into plasma;
- ✓ Antioxidant enzymes and Heat Shock protein expression in PBMC;
- ✓ Antioxidant enzymes and Heat Shock proteins as EVs cargo;
- ✓ Levels of lipid peroxidation (4-HNE) and protein carbonylation (PCO) in EVs, PBMCs or plasma.

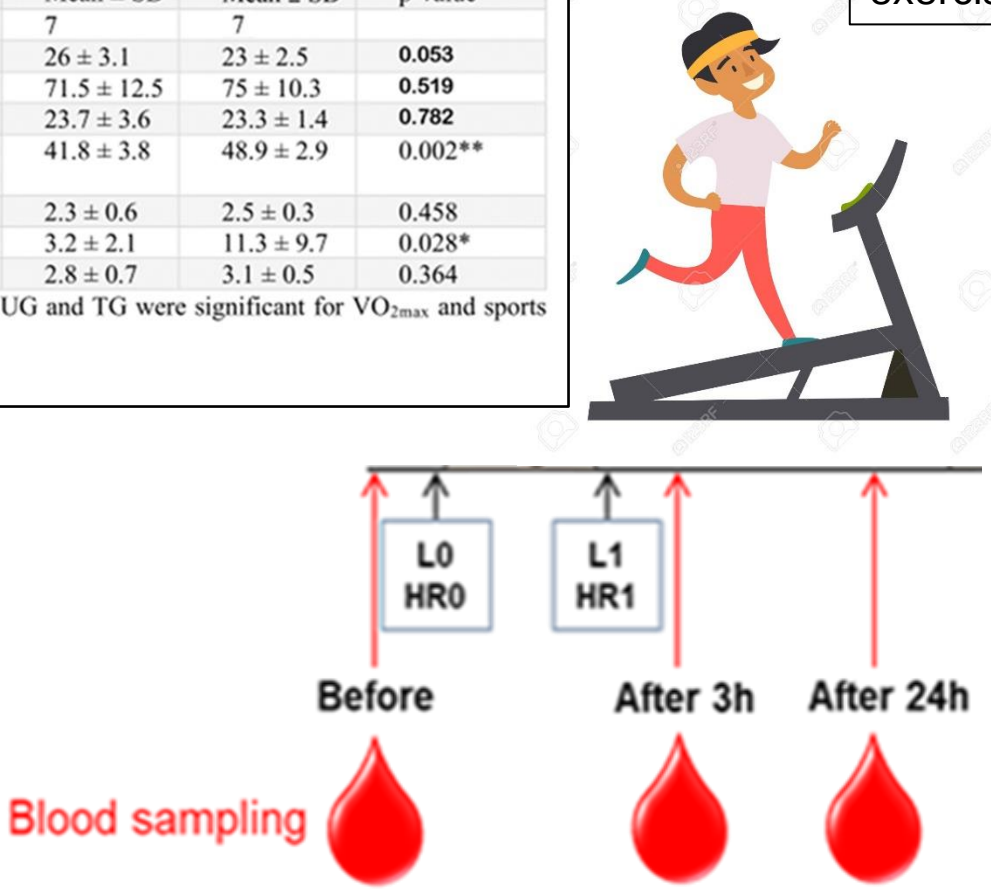
Material and methods

Table 1: Participant Baseline Characteristics

Subject Characteristics	UTS	TS	
	Mean ± SD	Mean ± SD	p-value
N. Subjects	7	7	
Age (yrs)	26 ± 3.1	23 ± 2.5	0.053
Weight (kg)	71.5 ± 12.5	75 ± 10.3	0.519
BMI	23.7 ± 3.6	23.3 ± 1.4	0.782
VO_{2max} (ml/kg/min)	41.8 ± 3.8	48.9 ± 2.9	0.002**
Work Index	2.3 ± 0.6	2.5 ± 0.3	0.458
Sport Index	3.2 ± 2.1	11.3 ± 9.7	0.028*
Leisure Index	2.8 ± 0.7	3.1 ± 0.5	0.364

Differences between UG and TG were significant for VO_{2max} and sports index ($p \leq 0.05$) *

Participants: 14 healthy young males
Training session (EX): 30' of treadmill exercise at 70% HRmax

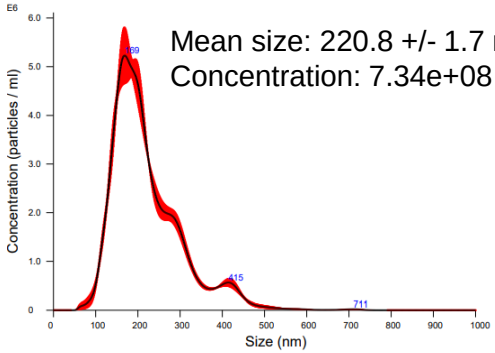


PBMC and plasma EVs isolation

Results 1:NanoSight analysis of plasma EVs released before (PRE) and 3h (POST 3H) after EX

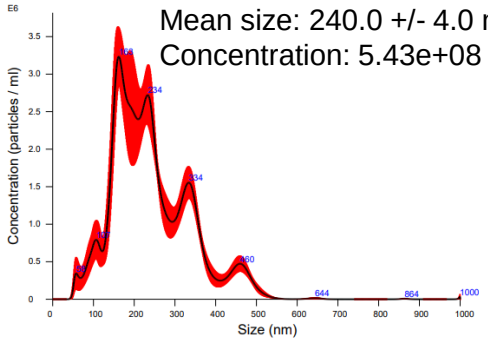
UTS

PRE



Mean size: 220.8 +/- 1.7 nm
Concentration: 7.34e+08 +/- 1.83e+07 particles/ml

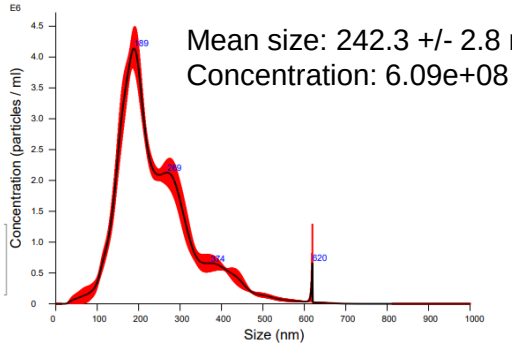
POST 3H



Mean size: 240.0 +/- 4.0 nm
Concentration: 5.43e+08 +/- 2.48e+07 particles/ml

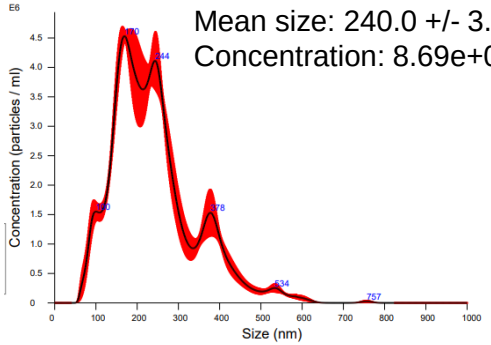
TS

PRE



Mean size: 242.3 +/- 2.8 nm
Concentration: 6.09e+08 +/- 3.14e+07 particles/ml

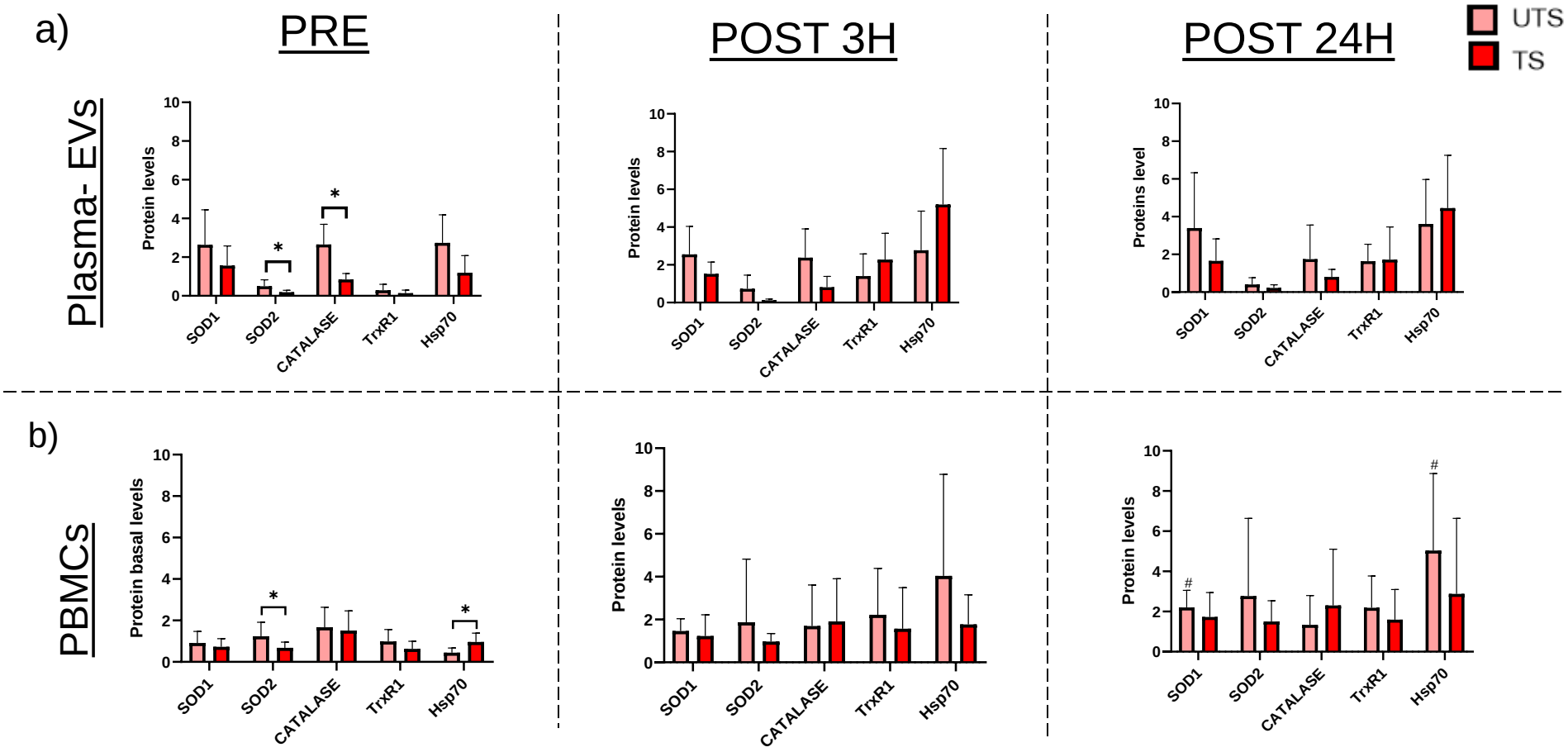
POST 3H



Mean size: 240.0 +/- 3.0 nm
Concentration: 8.69e+08 +/- 3.69e+07 particles/ml

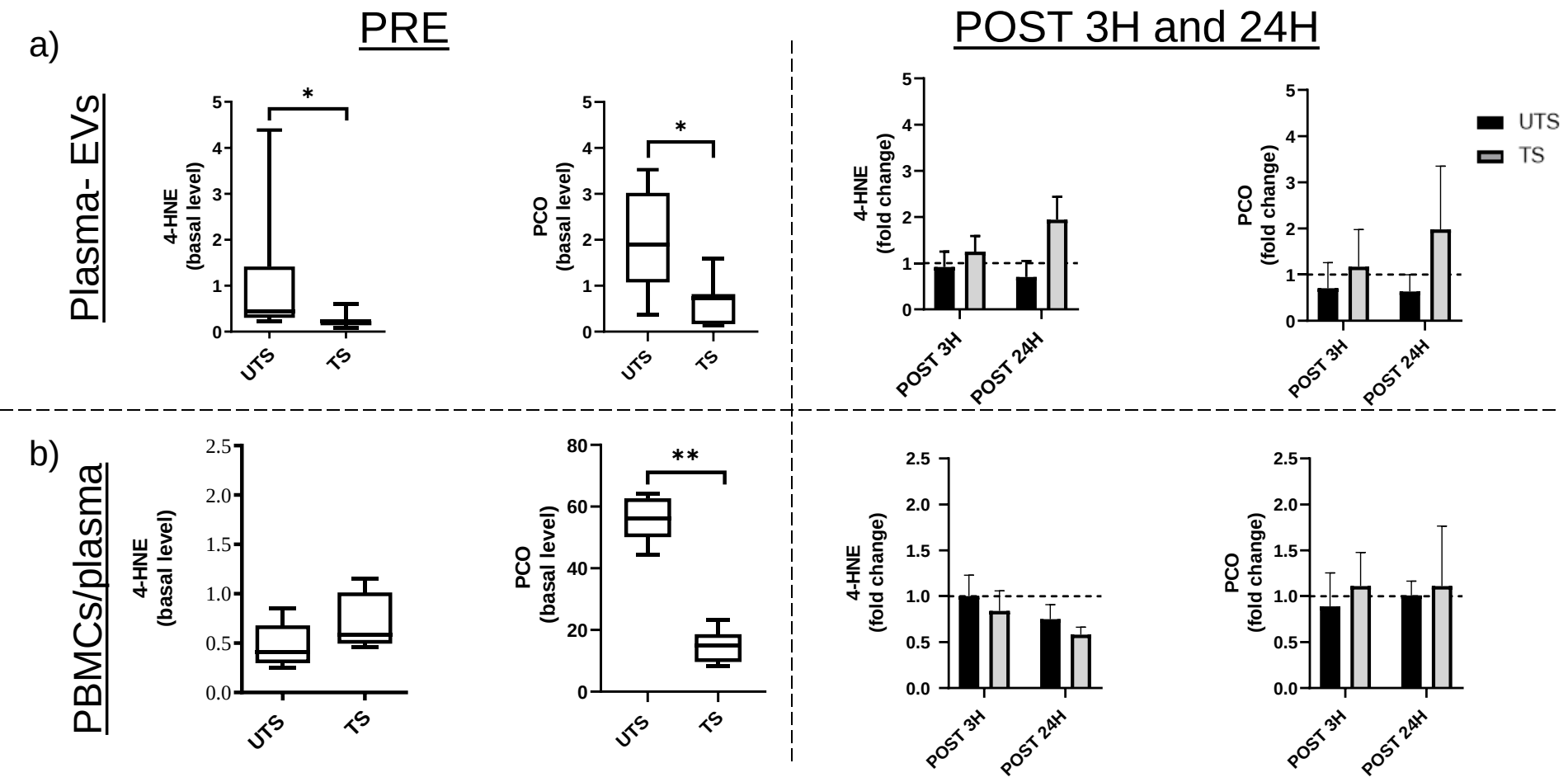
At baseline (PRE), NanoSight analysis shows a higher concentration of small plasma EVs in UTS compared with TS. At 3h post-acute exercise, the two experimental groups show an opposite trend of EVs concentration, with a decrease of EVs content in UTS and an increase in TS. Furthermore, in UTS the EVs were larger in size at post 3h when compared with their basal levels (PRE).

Results 2: EVs cargo and PBMC protein expression analysis: TS vs UTS



a) In plasma EVs, at basal levels, UTS shows significantly higher levels of SOD2 ($p<0,05$) and CATALASE ($p<0,05$) than in TS, while there is no significant modulation due to EX. b) In PBMCs there is a different expression of SOD2, which is more expressed in UTS ($p<0,05$), while Hsp70 is more expressed in TS ($p<0,05$). 24h after EX there is an increase of SOD1 and Hsp70 in UTS with respect to basal levels (# $p<0,05$).

Results 3: 4-HNE and PCO in EVs and PBMCs/plasma: TS vs UTS



a) 4-HNE and PCO in EVs show significant differences between the 2 groups only at basal levels ($p<0,05$).
b) In PBMCs/plasma the difference between the 2 groups is significant only for PCO, which is higher in UTS ($p<0,005$).

Discussion and conclusion

- A single bout of acute endurance exercise can change the amount and size distribution of EVs released into circulation as a function of subjects' fitness levels. Indeed at 3h after acute exercise, plasma EVs show a decrease in the concentration in UTS with respect to their basal levels while there is no noteworthy change in TS.

- Plasma EVs shuttle functional molecules such as SOD1, SOD2, Catalase, TxrR1 and Hsp70, as well as 4-HNE and PCO also detected in PBMCs, before and after the acute exercise in the two groups of subjects. In particular, SOD2 and PCO show a similar modulation in EVs and PBMCs at baseline, where their levels are higher in UTS.

- Interpreting these results, it is reasonable to speculate that, in UTS, acute exercise encourages a greater up-take of EVs enriched in molecules involved in the stress response and signaling, so to guarantee a higher antioxidant capacity on a systemic level. On the other hand, in TS subjects, whose tissues are expected to have a larger antioxidant capacity than UTS, the greater presence of EVs in plasma after the acute bout of exercise (POST 3h), could be due to either a higher release or lower uptake of EVs into tissues. Therefore, depending on the systemic state, exercise-induced EVs may be unused or absorbed based on the ability of specific tissues to respond to stress. However, further studies are needed to demonstrate the biological significance of these results.

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