Manuscript Title: Pulmonary ventilation and gas exchange during prolonged exercise in humans: influence of dehydration, hyperthermia and sympathoadrenal activity

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Underlying hypotheses: We hypothesised that (1) compensatory adjustments in pulmonary gas exchange would occur during prolonged intense exercise in the heat, such that arterial blood gases and acid-base balance disturbances are minimised, (2) hyperthermia, but not dehydration, independently would increase ventilation during prolonged intense exercise, and (3) adrenaline infusion during prolonged exercise in the heat would significantly increase ventilation

Abbreviations

ABE, actual base excess; $\mathbf{a} \cdot \mathbf{\bar{v}} O_2$ diff, arterial mixed-venous oxygen content difference; $C_a O_2$, arterial oxygen content; $C \mathbf{\bar{v}} O_2$, mixed-venous oxygen content; $C_{\mathbf{\bar{r}v}} O_2$, femoral venous oxygen content; $C \mathbf{\bar{v}} C O_2$, mixed-venous carbon dioxide content; $C_{\mathbf{fv}} C O_2$, femoral venous oxygen content; $C \mathbf{\bar{v}} C O_2$, mixed-venous carbon dioxide content; f_b , breathing frequency; $[Hb]_a$, arterial blood haemoglobin concentration; $[NA]_a$, arterial noradrenaline concentration; $[A]_a$, arterial adrenaline concentration; $P_a O_2$, arterial partial pressure of oxygen; $P_a C O_2$, arterial partial pressure of carbon dioxide; $P \mathbf{\bar{v}} C O_2$, mixed-venous partial pressure of carbon dioxide; \mathbf{Q} , cardiac output; $S_a O_2$, arterial oxygen saturation; T_c , core (oesophageal) temperature; T_{sk} , mean skin temperature; V_D , anatomical dead space; \dot{V}_E , pulmonary ventilation; \dot{V}_A , alveolar ventilation; $\dot{V}_A/\dot{\mathbf{Q}}$, ventilation-perfusion ratio; V_T , tidal volume; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide output; $\mathbf{\bar{v}}-aCO_2$ diff, mixed-venous arterial CO₂ content difference.

Definitions of 'n'

Question 1: n = number of ventilatory and systemic blood flow responses across time, hydration conditions and total comparisons Question 2: n = number of blood gases and acid-base balance responses across time, hydration conditions and total comparisons Question 3: n = number of blood contents, a- \bar{v} differences, $\dot{V}O_2$ and $\dot{V}CO_2$ across time, hydration conditions and total comparisons Question 4: n = number of systemic and leg blood gas contents, $\dot{V}CO_2$ and $\dot{V}O_2$ across time, hydration conditions and total comparisons Question 5: n = number of data points compared in the regression analysis Question 6: n = number of participants per comparison

Question 7: n = number of ventilatory responses across time, infusion type (adrenaline vs. saline) and total comparisons

Question 8: n = number of data points compared in the regression analysis

Question 9: n = number of temperature responses across time, hydration conditions and total comparisons Question 10: n = number of blood volume and osmolality responses across time, hydration conditions and total comparisons Question 11: n = number of expiratory and mixed venous gases responses across time, hydration conditions and total comparisons Question 12: n = number of catecholamine responses across time, hydration conditions and total comparisons Question 13: n = number of data points in the regression analysis Question 14: n = number of participants per comparison Question 15: n = number of participants per comparison Question 16: n = number of participants per comparison Question 17: n = number of participants per comparison Question 18: n = number of participants per comparison Question 19: n = number of participants per comparison Question 20: n = number of participants per comparison Question 21: n = number of participants per comparison Question 22: n = number of participants per comparison Question 23: n = number of participants per comparison Question 24: n = number of participants per comparison Question 25: n = number of participants per comparison Question 26: n = number of participants per comparison Question 27: n = number of participants per comparison Question 28: n = number of participants per comparison Question 29: n = number of temperature responses across time, infusion type (adrenaline vs. saline) and total comparisons Question 30: n = number of $\dot{V}O_2$ and $\dot{V}CO_2$ responses across time, infusion type (adrenaline vs. saline) and total comparisons

Question 31: n = number of $P_{ET}CO_2$ responses across time, infusion type (adrenaline vs. saline) and total comparisons

Question 32: n = number of catecholamine responses across time, infusion type (adrenaline vs. saline) and total comparisons

Statistical summary table

Experimental question #	Finding/ conclusion	Experimental variable & units	Statistical test	Mean value	SD	n	Data comparisons	Main & interaction effects P**	Post-hoc results P**	Figure/ table comments
1. What are the ventilatory	$V_{E,} f_{b,} V_{T,} VA,$ Q and VA/Q	V _E , I/min	Two-way ANOVA (time	n/a	n/a	5	Time	0.0204	Time vs. 20 min – DH (*) 60, 90, 120 & 134	Figure 1
and systemic blood flow	exhibited significant		x condition) with repeated			2	Hydration	<0.0001	min: 0.0113; 0.0072; 0.0473; 0.0060	N = 7 participants
responses to prolonged exercise with progressive dehydration	differences overtime in the dehydration and		measures with Bonferroni post-hoc analysis			10	Time x Hydration	0.0030	Time vs 20 min – Control (*) 90, 120, 134 min: 0.0008; 0.0114;	, except for VA and VA/Q N = 6 participants
and hyperthermia and	hyperthermia condition (DH) and in								0.0033 DH vs control (†)	Overtime differences were
maintained	very few								134 min: 0.0042	compared
euhydration	cases in the	<i>f</i> _b , breaths/min				5	Time	0.0001	Time vs 20 min – DH	to 20 min of
control?	euhydration control					2	Hydration	0.0874	(*) 90, 120 & 134 min: 0.0049; 0.0569;	exercise.
	condition.					10	Time x Hydration	0.0035	0.0025	
	There were significant interactions between time								Time vs 20 min – Control (*) 90 min: 0.0445	
	and hydration								DH vs control (†) 134 min: 0.0042	
	conditions in	V _T , I				5	Time	0.8225	Time vs 20 min – DH	
	all variables (Time x					2	Hydration	0.0599	(*) NS	
	Hydration					10	Time x Hydration	0.1334		

interactions), except V_T .					Time vs 20 min – Control (*) NS
					DH vs control (†) NS
	V _A , I/min	5	Time	<0.0001	Time vs 20 min – DH
		2	Hydration	0.0009	(*) 60, 90, 120 & 134 min: 0.0052; 0.0089;
		10	Time x Hydration	<0.0001	0.0163; 0.0045
					Time vs 20 min – Control (*) 60, 90, 120, 134 min: 0.0052; 0.0091; 0.0444; 0.0108
					DH vs control (†) 120, 134 min: 0.0192; 0.0016
	Q, I/min	5	Time	>0.0001	Time vs 20 min – DH
		2	Hydration	0.0086	(*) 120 & 134 min: 0.0143; 0.0038
		10	Time x Hydration	>0.0001	Time vs 20 min – Control (*) NS
					DH vs control (†) 90, 120 & 134 min: 0.0486; 0.0009; 0.0010
	VA/Q ratio	5	Time	>0.0001	Time vs 20 min – DH
		2	Hydration	0.0007	(*)

Experimental Physiology

						10	Time x Hydration	>0.0001	90, 120 & 134 min: 0.0176; 0.028; 0.0002 Time vs 20 min – Control (*) NS DH vs control (†) 90, 120 & 134 min: 0.0302; 0.0045; >0.0001	
2. What are	PaO ₂ ,	PaO ₂ , mmHg	Two-way	n/a	n/a	5	Time	0.0154	Time vs 20 min – DH	Figure 2
the blood	PaCO ₂ ,		ANOVA (time			2	Hydration	0.0046	(*)120 & 134 min:	
gases and acid-base	[Hb] _a , pH _a and [HCO ₃ -] _a		x condition) with repeated			10	Time x Hydration	0.0064	0.0086; 0.0074	N = 7 participants
balance	exhibited		measures with						Time vs 20 min –	
responses to	significant		Bonferroni						Control (*)	Overtime
prolonged exercise with	differences overtime in		post-hoc analysis						NS	differences were
progressive	the		anarysis						DH vs control (†)	compared
dehydration	dehydration								120 & 134 min:	to 20 min of
and	and								0.0128; 0.0024	exercise.
hyperthermia	hyperthermia	PaCO ₂ , mmHg				5	Time	<0.0001	Time vs 20 min – DH	
and	condition					2	Hydration	<0.0031	(*)120 & 134 min:	
maintained euhydration	(DH) and in very few					10	Time x Hydration	0.0270	0.0017; 0.0004	
control?	cases in the								Time vs 20 min –	
	euhydration								Control (*)	
	control								NS	
	condition. Yet, SaO ₂								Dilling control (1)	
	and ABE_a								DH vs control (†) 60, 90, 120 & 134 min:	
	were								0.0384; 0.0092;	
	unchanged.								0.01022; 0.0057	
		SaO ₂ , %				5	Time	0.1490	Time vs 20 min – DH]
						2	Hydration	0.0228	NS	

There were significant interactions between time and hydration		10	Time x Hydration	0.4366	Time vs 20 min – Control (*) NS DH vs control (†)
conditions in					NS
most	[Hb] _a , g/l	5	Time	0.0474	Time vs 20 min – DH
variables		2	Hydration	0.0129	(*) NS
(Time x Hydration interactions), except SaO ₂ , pH_a and ABE _a		10	Time x Hydration	0.0007	Time vs 20 min – Control (*) NS DH vs control (†) 60, 90, 120 & 134 min: 0.0318; 0.0125;
					0.0048; 0.0049
	pHa	5	Time	<0.0001	Time vs 20 min – DH
		2	Hydration	0.0061	(*) 134 min: 0.0076
		10	Time x Hydration	0.1664	Time vs 20 min – Control (*) NS
					DH vs control (†) 134 min: 0.0353
	[HCO ₃ -] _a ,	5	Time	0.0034	Time vs 20 min – DH
	mmol/l	2	Hydration	0.0220	(*) NS
		10	Time x Hydration	0.0064	Time vs 20 min – Control (*) NS
					DH vs control (†) 134 min: 0.0029
	ABE _a , mmol/l	5	Time	0.6544	Time vs 20 min – DH
		2	Hydration	0.8666	(*) NS
		10	Time x Hydration	0.2477	

									Time vs 20 min – Control (*) NS DH vs control (†) NS	
3. What are the blood gas contents, a-v difference, VO ₂ and VCO ₂ responses to prolonged exercise with	$\begin{array}{c} CaCO_2,\\ C\overline{v}CO_2,\ \overline{v}-\\ aCO_2\ diff,\\ VCO_2,\ CaO_2,\\ C\overline{v}O_2,\ a-\overline{v}O_2\\ diff\ and\ VO_2\\ exhibited\\ significant \end{array}$	CaCO ₂ , ml/l	Two-way ANOVA (time x condition) with repeated measures with Bonferroni post-hoc analysis	n/a	n/a	5 2 10	Time Hydration Time x Hydration	0.0141 0.0163 0.0062	Time vs 20 min – DH (*) 134 min 0.0485 Time vs 20 min – Control (*) NS DH vs control (†) 120 & 134 min:	Figure 3 N = 7 participants Overtime differences were
progressive dehydration and hyperthermia and maintained euhydration control?	differences overtime in the dehydration and hyperthermia condition (DH) and in	C⊽CO₂, ml/l				5 2 10	Time Hydration Time x Hydration	0.0142 0.6321 0.1518	0.0021; 0.0035 Time vs 20 min – DH (*) NS Time vs 20 min – Control (*) NS DH vs control (†)	compared to 20 min of exercise.
	very few cases in the euhydration control condition. There were significant interactions between time	⊽-aCO₂ diff, ml/l				5 2 10	Time Hydration Time x Hydration	0.0006 0.0032 <0.0001	NS Time vs 20 min – DH (*) 120 & 134 min 0.0187; 0.0017 Time vs 20 min – Control (*) NS DH vs control (†) 90, 120 & 134 min:	
	and hydration conditions in all variables (Time x	VCO ₂ , I/min	-			5 2 10	Time Hydration Time x Hydration	0.0006 0.0365 0.0058	0.0440; 0.0025; <0.0001 Time vs 20 min – DH (*) NS	

Hydration interactions), except					Time vs 20 min – Control (*) NS
C⊽CO ₂					DH vs control (†) 134 min: 0.0058
	CaO ₂ , ml/l	5 2 10	Time Hydration Time x Hydration	0.0001 0.0131 <0.0001	Time vs 20 min – DH (*) 120 & 134 min: 0.0144; 0.0098
					Time vs 20 min – Control (*) NS DH vs control (†) 134 min: 0.0398
	C⊽O₂, ml/l	5 2 10	Time Hydration Time x Hydration	0.0001 0.0331 <0.0001	Time vs 20 min – DH (*) 120 & 134 min: 0.0309; 0.0096 Time vs 20 min –
					Control (*) NS DH vs control (†) 120 & 134 min: 0.0008; 0.0024
	a-⊽O₂ diff, ml/l	5 2 10	Time Hydration Time x Hydration	0.0241 0.0149 <0.0001	Time vs 20 min – DH (*) 90, 120 & 134 min: 0.0257; 0.0013; 0.0005
					Time vs 20 min – Control (*) NS DH vs control (†)
					120 & 134 min: 0.0013; 0.0017

		VO ₂ , I/min				5	Time	<0.0001	Time vs 20 min – DH (*) 120 & 134 min: 0.0168; 0.0060	
						2	Hydration	0.4692	Time vs 20 min –	
						10	Time x Hydration	0.0028	Control (*) 90, 120 & 134 min: 0.0011; 0.0014; 0.0007	
									DH vs control (†) NS	
4. What are	C⊽CO₂,	C⊽CO₂, ml/l	Two-way	n/a	n/a	5	Time	0.0622	Time vs 20 min – DH	Figure 4
the systemic	CfvCO ₂ ,		ANOVA (time			2	Hydration	0.6321	(*) NS	
and leg blood gas contents, VCO ₂ and VO ₂	$C\overline{v}O_2$, $CfvO_2$, VCO ₂ and VO ₂		x condition) with repeated measures with			10	Time x Hydration	0.1518	Time vs 20 min – Control (*) NS	N = 7 participants
responses to prolonged exercise with	exhibited significant differences		Bonferroni post-hoc analysis						DH vs control (†) NS	Overtime differences were
progressive	overtime in	CfvCO ₂ , ml/l				5	Time	0.0022	Time vs 20 min – DH	compared
dehydration	the					2	Hydration	0.6372	(*) NS	to 20 min of
and hyperthermia and maintained	dehydration and hyperthermia condition					10	Time x Hydration	0.1574	Time vs 20 min – Control (*) NS	exercise.
euhydration control?	(DH) and in very few								DH vs control (†) NS	
	cases in the	C⊽O₂, ml/l				5	Time	<0.0001	Time vs 20 min – DH	
	euhydration					2	Hydration	0.9733	(*)120 & 134 min:	
	control condition.					10	Time x Hydration	<0.0001	0.0166; 0.0049	
	There were significant								Time vs 20 min – Control (*) NS	
	interactions								DH vs control (†)	

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	between time and							120 & 134 min: 0.0301; 0.0227
	hydration	CfvO ₂ , ml/l			5	Time	<0.0001	Time vs 20 min – DH
	conditions in				2	Hydration	0.5383	(*) NS
	all variables				10	Time x Hydration	0.7370	
	(Time x							Time vs 20 min –
	Hydration							Control (*) NS
	interactions).							
	conditions in many							DH vs control (†) NS
	variables,	Systemic		_	5	Time	0.0006	Time vs 20 min – DH
	except	VCO ₂ , I/min			2	Hydration	0.0365	(*) NS
	C⊽CO _{2,}				10	Time x Hydration	0.0058	
	$CfvCO_2$ and							Time vs 20 min –
	CfvO ₂							Control (*) NS
								DH vs control (†)
								134 min: 0.0058
		Legs VCO ₂ ,		-	5	Time	0.8053	Time vs 20 min – DH
		I/min		-	2	Hydration	0.7708	(*) NS
				_	10	Time x Hydration	0.3314	
						,		Time vs 20 min –
								Control (*) NS
								DH vs control (†)
		Ounterrain V/O		-	_	Time	-0.0001	NS Time vs 20 min – DH
		Systemic VO ₂ , I/min		-	5 2	Time	<0.0001 0.4692	(*) 120 & 134 min:
		1/11111		-	10	Hydration Time x Hydration	0.4692	0.0168; 0.0060
					10	Time x Hydration	0.0028	0.0108, 0.0000
								Time vs 20 min –
								Control (*) 90, 120 &
								134 min: 0.0011;
								0.0014; 0.0007
								DH vs control (†)
			1 1			1	1	

the ventilatory induced significant AVE, MININ Otherway 5.0 2.2 22 DH VS. control 0.001 IVA Pigure Figure 10 and 1	5.What are the relationships between VE and f_b vs. core temperature and arterial catecholamine s during prolonged exercise with progressive dehydration and hyperthermia (DH) and maintained euhydration control (control)?	There were significant direct relationships between increases in V_E and fb with the increases in core temperature and combined catecholamin es	Legs VO ₂ , I/min V _E vs. T _c , I/min & °C f _b vs. T _c , breaths/min & °C VE vs. arterial catecholamine s, I/min & mmol/I f _b vs. arterial catecholamine s, breaths/min & mmol/I	Regression analysis	n/a	n/a	5 2 10 5 5 5 5 5 5 5 5 5 5 5 5	Time Hydration Time x Hydration DH Control DH Control DH Control DH Control	0.0251 0.8373 0.1576 R ² = 0.994 R ² = 0.818 R ² = 0.982 R ² = 0.982 R ² = 0.972 R ² = 0.973 R ² = 0.898	NS Time vs 20 min – DH (*) NS Time vs 20 min – Control (*) NS DH vs control (†) NS P = 0.0002 P = 0.0010 P = 0.0011 P = 0.0012 P = 0.0011 P = 0.0012 P = 0.012 P = 0.014	Figure 5 DH vs. control, 22 participants
			ΔV_{E} , l/min				-			n/a	Figure 6
combined increases in $\Delta f_{\rm b}$, repeated 4 3 22 DH vs. control 0.0001 control		0	Λ f.							-	control, 22
										4	participants
separate but not VT. In Bonferroni 2.4 3 8 D vs. control 0.005 H vs.	· /	,	broattio/ffill				-			4	
			$\Lambda / = m $							4	control, 7
		· ·	Δv_{T} , III	•						4	participants

hyperthermia (H)?	these responses.			175	200	8	D vs. control	0.001		D vs. control, 8 participants															
7. What are	VE, <i>f</i> _b , V _T ,	V _E , I/min	Two-way (time	n/a	n/a	8	Time	<0.0001	Time vs 10 min – A	Figure 7															
the ventilatory	exhibited		x condition)			2	Infusion	0.0140	infusion (*)																
responses to adrenaline (A) and saline (S) infusion during prolonged exercise with progressive dehydration and hyperthermia?	significant differences overtime in the adrenaline infusion (A and some in the control saline (S) control condition. Time (min) x		ANOVA with repeated measures with Bonferroni post-hoc analysis			16	Time x Infusion	0.0016	45, 85 & 120 min: 0.0016; 0.0269; 0.0252 Time vs 10 min – S infusion (*) 45, 85 & 120 min: 0.0122; 0.0674; 0.0282 A vs S control (†) 45, 85 & 120 min: 0.0063; 0.0077; 0.0672	N= 7 participants															
	condition (A	<i>f</i> _b , breaths/min				8	Time	<0.0001	Time vs 10 min – A	-															
	vs. S control)					2	Infusion	0.1214	infusion (*)																
	interactions. There was a significant interaction					16	Time x Infusion	0.1546	45, 85 & 120 min: 0.0196; 0.0201; 0.0130																
	between time and conditions in VE, but not in f_b and V _T .								Time vs 10 min – S infusion (*) 45, 85 & 120 min: 0.0123; 0.0029; 0.0068 A vs S control (†) NS																
		V _T , ml				8	Time	0.1471	Time vs 10 min – A infusion (*)																
																					2	Infusion	0.2891	NS	
							Time x Infusion	0.0733	7																

8. What are	There were	V vo T	Pagrossian	n/c	n/c		Adrenaline	R ² =0.990	Time vs 10 min – S infusion (*) NS A vs S control (†) NS P = 0.004	
8. What are the	i nere were significant	V _E vs. T _c , I/min & °C	Regression analysis	n/a	n/a	4	Saline	R ² =0.990 R ² =0.996	P = 0.004 P = 0.001	Figure 8
relationships	direct	$f_{\rm b}$ vs. Tc,	anarysis			4	Adrenaline	R ² =0.996 R ² =0.983	P = 0.001 P = 0.008	N = 7
between VE	relationships	breaths/min &				4				participants
and <i>f</i> _b vs. core	between	°C				4	Saline	R ² =0.970	P = 0.014	P P
temperature and arterial	increases in V _E and fb	V _E vs. arterial catecholamine				4	Adrenaline	R ² =0.986	P = 0.006	
catecholamine s with	with the increases in	s, l/min & mmol/l				4	Saline	R ² =0.932	P = 0.034	
adrenaline (A) and saline (S) infusion during	core temperature and	<i>f</i> _b vs. arterial catecholamine				4	Adrenaline	R ² =0.950	P = 0.024	
prolonged exercise with dehydration & hyperthermia?	combined catecholamin es	s, breaths/min & mmol/l				4	Saline	R ² =0.969	P = 0.015	
9. What are the effects of	Dehydration	T _c , °C	Two-way (time	n/a	n/a	5	Time	<0.0001	Time vs 20 min – DH	Table 2
dehydration on	significantly increase T _c		x condition) ANOVA with			2	Hydration	0.0021	(*) 90, 120 & 134 min; 0.0531; 0.0047;	N = 7
core and skin temperature	after 90 min of exercise		repeated measures with			10	Time x Hydration	<0.0001	0.0005	participants
during prolonged exercise	while T _{sk} remained unchanged.		Bonferroni post-hoc analysis						Time vs 20 min – Control (*) NS	Overtime differences were
compared to euhydration control?									DH vs control (†) 90, 120 & 134 min: 0.0084; 0.0005; 0.0002	compared to 20 min of exercise.
		T _{sk} , °C				5	Time	0.0003	Time vs 20 min – DH (*) NS	

						2	Hydration	0.0365		
						10	Time x Hydration	0.8446	Time vs 20 min – Control (*) NS	
									DH vs control (†) NS	
10. What are the effects of	BV & osmolality	Blood volume, I	Two-way (time x condition)	n/a	n/a	5	Time	0.0065	Time vs 20 min – DH (*)134 min; 0.0487	Table 2
dehydration on	exhibited		ANOVA with			2	Hydration	0.0027		N = 7
blood volume and osmolality	significant differences		repeated measures with			10	Time x Hydration	0.0004	Time vs 20 min – Control (*) NS	participants
during prolonged exercise compared to euhydration	overtime in the dehydration and hyperthermia		Bonferroni post-hoc analysis						DH vs control (†) 60, 90, 120 & 134 min: 0.0306; 0.0018; 0.0006; 0.0004	Overtime differences were compared to 20 min of
control?	condition (DH) and	Osmolality, mOsm/kg				5	Time	0.5785	Time vs 20 min – DH (*) 60, 90, 120 & 134	exercise.
	some in the control					2	Hydration	0.0003	min: 0.0075; 0.0168; 0.0015; 0.0035	
	condition.					10	Time x Hydration	<0.0001		
	Time (min) x condition (DH vs.								Time vs 20 min – Control (*) 134 min: 0.0059	
	control) interactions. There were significant interactions								DH vs control (†) 60, 90, 120 & 134 min: 0.0043; 0.0002; 0.0002; 0.0001	
	between time and conditions in									
	both variables									

11. What are the effects of dehydration and hyperthermia (DH) on end- tidal gases during prolonged exercise compared to euhydration control?	P _{ET} CO ₂ , $P\overline{V}O_2$ & $P_{ET}O_2$ exhibited significant differences overtime in the dehydration and hyperthermia condition (DH) and some in the control condition. Time (min) x condition (DH vs. control) interactions. There were significant interactions between time and conditions in $P_{ET}CO_2$ and $P_{ET}O_2$ but not in $P\overline{V}O_2$. Plasma	P _{ET} CO ₂ , mmHg PVO ₂ , mmHg PETO ₂ , mmHg	Two-way (time x condition) ANOVA with repeated measures with Bonferroni post-hoc analysis	n/a	n/a n/a	5 2 10 5 2 10 5 5 2 10	Time Hydration Time x Hydration Time Hydration Time x Hydration Time Hydration Time x Hydration	0.0013 0.0320 0.0054 0.0054 0.0029 0.0318 0.2565 0.2565 0.0031 0.0618 0.0618 0.0448	Time vs 20 min – DH (*) 60, 90, 120 & 134 min: 0.0300; 0.0003; 0.0067; 0.0253 Time vs 20 min – Control (*)60, 90, 120 & 134 min: 0.0299; 0.0096; 0.0043; 0.0040 DH vs control (†) 120 & 134 min: 0.0043; 0.0040 Time vs 20 min – DH (*) 120 & 134 min: 0.0114; 0.0315 Time vs 20 min – Control (*) 120 & 134 min: 0.0015; 0.0484 DH vs control (†) NS Time vs 20 min – DH (*) 60, 90, 120 & 134 min: 0.0015; 0.0484 DH vs control (†) NS Time vs 20 min – DH (*) 60, 90, 120 & 134 min: 0.0028; 0.0043; 0.0156; 0.0500 Time vs 20 min – Control (*) NS DH vs control (†) NS DH vs control (†) 134 min: 0.0311 Time vs 20 min – DH	Table 2 N = 7 participants Overtime differences were compared to 20 min of exercise.
the effects of	catecholamin		x condition)						(*) 60, 90, 120 & 134	

dehydration on	es exhibited		ANOVA with			2	Hydration	0.0076	min: 0.0075; 0.0011;	N = 7
circulating	significant		repeated			10	Time x Hydration	<0.0001	0.0001; 0.0007	participants
catecholamine	differences		measures with							
s during	overtime in		Bonferroni						Time vs 20 min –	Overtime
prolonged	the		post-hoc						Control (*) NS	differences
exercise	dehydration		analysis							were
compared to	and								DH vs control (†)	compared
euhydration	hyperthermia								60,90, 120 134 min:	to 20 min of
control?	condition								0.0247; 0.0417;	exercise.
	(DH) and								0.0049; 0.0012	
	some in the	[A], mmol/l				5	Time	<0.0001	Time vs 20 min – DH	
	control								(*) 134 min: 0.0466	
	condition									
						2	Hydration	0.0040	Time vs 20 min –	
	Time (min) x					10	Time x Hydration	0.1401	Control (*) NS	
	condition									
	(DH vs.								DH vs control (†)	
	control)								90, 120 134 min:	
	interactions.								0.0177; 0.0445;	
	There were								0.0313	
	significant									
	interactions									
	between time									
	and									
	conditions in									
	[NA] but not									
	[A].									
13. What are	DH induced	T _c control, °C	One-way	38.2	0.4	22	DH vs control	<0.0001	n/a	Table 3
the effects of	significant	T _c DH, °C	ANOVA	39.1	0.4	22	-			
combined	increases in	T _{sk} control, °C	(condition)	34.2	0.6	22	-	0.0021		DH vs.
dehydration	T _c with		with repeated	J4.2	0.0	~~		0.0021		control, 22
and	smaller		measures with							participants
hyperthermia	increases in		Bonferroni	047	0.0	00	4			
(DH) on body	T _{sk} compared	T _{sk} DH, ⁰C	post-hoc	34.7	0.9	22				
temperatures	to control.		analysis							1

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during prolonged exercise compared to euhydration control? 14. What are	DH induced	%BM loss	One-way	-0.5	0.3	22	DH vs control	<0.0001	n/a	Table 3
the effects of	significant	control	ANOVA					20.0001	174	
combined dehydration	increases in %BM loss,	%BM loss DH	(condition) with repeated	-4.6	0.5	22				DH vs. control, 22
and hyperthermia	[Hb] and osmolality	[Hb] control, g/l	measures with Bonferroni	159	8	22		<0.0001		participants
(DH) on % body mass loss,	compared to control.	[Hb] DH, g/l	post-hoc analysis	168	10	22				
haemoglobin and blood osmolality		Osmolality control, mOsm/kg		278	4	22		<0.0001		
during prolonged exercise compared to euhydration control?		Osmolality DH, mOsm/kg		298	4	22				
15. What are the effects of combined	DH did not significantly alter VO ₂ or	VO₂ control, I/min	One-way ANOVA (condition)	3.09	0.25	22	DH vs control	0.1925	n/a	Table 3 DH vs.
dehydration	VCO ₂ compared to	VO₂ DH, I/min	with repeated measures with	3.10	0.26	22				control, 22 participants
hyperthermia (DH) on	control.	VCO ₂ control, I/min	Bonferroni post-hoc	2.94	0.26	22		0.0895		
metabolism during prolonged exercise compared to		VCO ₂ DH, I/min	analysis	2.97	0.26	22				

euhydration control?										
16. What are the effects of	DH significantly	V _E control, I/min	One-way ANOVA	68.6	6.2	22	DH vs control	<0.0001	n/a	Table 3
combined	increased $V_{E,}$	V _E DH, I/min	(condition)	73.5	6.9	22				DH vs.
dehydration and	$f_{\rm b}$ and $V_{\rm T}$, and tended	<i>f</i> _b control, breaths/min	with repeated measures with	34	6	22		<0.0001	_	control, 22 participants
hyperthermia (DH) on the	to increase P _{ET} CO ₂	<i>f</i> ₀ DH, breaths/min	Bonferroni post-hoc	38	7	22				
ventilatory responses	compared to control.	V _T control, I	analysis	2.05	0.24	22		0.0086		
during	control.	V⊤ DH, I		1.96	0.26	22				
prolonged exercise		P _{ET} CO ₂ control, mmHg		37	4	22		0.0605		
compared to euhydration control?		P _{ET} CO₂ DH, mmHg		35	4	22				
17. What are the effects of	As per design, H	T_c control, °C	One-way ANOVA	38.3	0.2	7	H vs control	0.0001	n/a	Table 3
isolated hyperthermia	induced significant	T₀ H, °C	(condition) with repeated	39.2	0.3	7				H vs. control, 7
(H) on body temperature	increases in T _c with	T_{sk} control, °C	measures with Bonferroni	34.0	0.6	7		0.0260		participants
responses during prolonged exercise compared to euhydration control?	smaller increases in T_{sk} compared to control.	T₅k H, °C	post-hoc analysis	34.6	0.8	7				
18. What are the effects of	As per design, H did	%BM loss control	One-way ANOVA	-0.5	0.3	7	H vs control	0.0707	n/a	Table 3
isolated hyperthermia	not	%BM loss H	(condition) with repeated	-4.6	0.5	7				H vs. control, 7
(H) on % body	change %BM	[Hb] control, g/l	measures with	159	8	7]	0.0488		participants

mass loss,	loss or	[Hb] H, g/l	Bonferroni	168	10	7				
haemoglobin and blood osmolality responses	osmolality compared to control.	Osmolality control, mOsm/kg	post-hoc analysis	278	4	7		0.2140	n	
during prolonged exercise compared to euhydration control?		Osmolality H, mOsm/kg		298	4	7				
19. What are the effects of	H did not alter VO ₂ but	VO ₂ control, I/min	One-way ANOVA	3.15	0.28	7	H vs control	0.3955	n/a	Table 3
isolated hyperthermia	reduced VCO ₂	VO ₂ H, I/min	(condition) with repeated	3.16	0.27	7				DH vs. control, 22
(H) on metabolism	compared to control.	VCO ₂ control, I/min	measures with Bonferroni	3.05	0.29	7		0.0222		participants
during prolonged exercise compared to euhydration control?		VCO₂ H, I/min	post-hoc analysis	2.93	0.21	7				
20. What are the effects of	DH significantly	V _E control, I/min	One-way ANOVA	68.5	4.9	7	H vs control	0.0007	n/a	Table 3
isolated	increased $V_{\text{E},}$	V _E H, I/min	(condition)	74.2	6.6	7				DH vs.
hyperthermia (H) on the	$f_{\rm b}$ and $P_{\rm ET}CO_2$ while	<i>f</i> _b control, breaths/min	with repeated measures with	35	6	7		0.0066		control, 22 participants
ventilatory responses	V⊤ remained unchanged	<i>f</i> ₀ H, breaths/min	Bonferroni post-hoc	38	6	7				
during	compared to	V⊤ control, I	analysis	2.00	0.25	7		0.7933		
prolonged	control.	V _T H, I		1.99	0.24	7				
exercise compared to		P _{ET} CO ₂ control, mmHg		38	4	7		0.0056		
euhydration control?		P _{ET} CO ₂ DH, mmHg		35	3	7				

21. What are the effects of	As per design, D did	T _c control, °C	One-way ANOVA	38.1	0.4	8	D vs control	0.0656	n/a n/a	Table 3
isolated dehydration	not increase T _c or T _{sk}	T _c D, °C	(condition) with repeated	38.2	0.3	8				D vs. control, 8
(D) on body temperature	compared to control.	T _{sk} control, °C	measures with Bonferroni	21.2	1.3	8		0.1266		participants
responses during prolonged exercise compared to euhydration control?		T₅k D, °C	post-hoc analysis	20.4	1.2	8				
22. What are the effects of	As per design, D	%BM loss control	One-way ANOVA	-0.1	0.2	8	D vs control	<0.0001	n/a	Table 3
isolated hyperthermia	reduced %BM loss	%BM loss D	(condition) with repeated	-4.2	0.3	8				D vs. control, 8
(D) on % body mass loss,	and increased	[Hb] control, g/l	measures with Bonferroni	156	8	8		<0.0001		participants
haemoglobin	[Hb] and	[Hb] D, g/l	post-hoc	164	7	8				
and blood osmolality responses	osmolality compared to control.	Osmolality control, mOsm/kg	analysis	281	3	8		<0.0001		
during prolonged exercise compared to euhydration control?		Osmolality D, mOsm/kg		296	5	8				
23. What are the effects of	D did not alter either	VO ₂ control, I/min	One-way ANOVA	3.22	0.34	8	D vs control	0.2662	n/a	Table 3
isolated dehydration	VO ₂ or VCO ₂ compared to	VO ₂ D, I/min	(condition) with repeated	3.20	0.34	8				D vs. control, 8
(D) on metabolism	control.	VCO ₂ control, I/min	measures with Bonferroni	3.04	0.34	8		0.2337		participants
during		VCO ₂ D, I/min		2.99	0.31	8				

prolonged exercise compared to euhydration control?			post-hoc analysis							
24. What are the effects of	D did not alter V_E , f_b or	V _E control, I/min	One-way ANOVA	67.5	5.8	8	D vs control	0.7194	n/a	Table 3
isolated	P _{ET} CO ₂ , but	V _E DH, I/min	(condition)	68.1	5.5	8				DH vs.
hyperthermia (H) on the	reduced V_T compared to	f _b control, breaths/min	with repeated measures with	30	4	8		0.0807		control, 22 participants
ventilatory responses	control.	<i>f</i> _b DH, breaths/min	Bonferroni post-hoc	32	4	8				
during		V _T control, I	analysis	2.31	0.47	8		0.0442		
prolonged exercise		V _T DH, I		2.14	0.32	8			-	
compared to		P _{ET} CO₂ control, mmHg		39	6	8		0.8994		
euhydration control?		P _{ET} CO₂ DH, mmHg		39	4	8				
25. What are the effects of	D+BV restoration	T _c control, °C	One-way ANOVA	38.0	0.4	8	D+BV restoration vs control	0.1705	n/a	Table 3
isolated dehydration	did not increase T _c	T _c D+BV restoration, °C	(condition) with repeated	38.1	0.3	8				D+BV vs. control, 8
and BV restoration	or T _{sk} compared to	T _{sk} control, °C	measures with Bonferroni	20.9	1.4	8		0.9762		participants
(D+BV restoration) on body temperature responses during prolonged exercise compared to	control.	T _{sk} D+BV restoration, °C	post-hoc analysis	20.9	0.9	8				
euhydration control?										

26. What are the effects of	As per design, D +	%BM loss control	One-way ANOVA	-0.3	0.2	8	D+BV restoration vs control	<0.0001	n/a	Table 3
isolated dehydration and BV	BV restoration reduced	%BM loss D+BV restoration	(condition) with repeated measures with	-4.2	0.4	8				D+BV vs. control, 8 participants
restoration (D+BV	%BM loss and [Hb]	[Hb] control, g/l	Bonferroni post-hoc	156	8	8		0.0221	-	P P
restoration) on % body mass	increased [Hb] while	[Hb] D+BV restoration, g/l	analysis	153	9	8				
loss, haemoglobin and blood	osmolality remained elevated	Osmolality control, mOsm/kg		281	3	8		0.0001		
osmolality responses during prolonged exercise compared to euhydration	compared to control.	Osmolality D+BV restoration, mOsm/kg		296	5	8				
control? 27. What are the effects of	D+BV restoration	VO ₂ control,	One-way ANOVA	3.21	0.34	8	D+BV restoration vs control	0.7560	n/a	Table 3
isolated dehydration and BV	did not alter VO ₂ but reduced	VO ₂ D+BV restoration, I/min	(condition) with repeated measures with	3.22	0.33	8				D+BV vs. control, 8 participants
restoration (D+BV	VCO ₂ compared to	VCO ₂ control, I/min	Bonferroni post-hoc	3.00	0.33	8		0.0065		
restoration) on metabolism during prolonged exercise compared to	control.	VCO ₂ D+BV restoration, I/min	analysis	2.95	0.31	8				
euhydration control?										

28. What are the effects of	D+BV restoration	V _E control, I/min	One-way ANOVA	65.5	6.0	8	D+BV restoration vs control	0.3689	n/a	Table 3
isolated dehydration and BV	did not alter V_E or $P_{ET}CO_2$, but	V _E D+BV restoration, I/min	(condition) with repeated measures with	66.6	4.9	8				D+BV vs. control, 8 participants
restoration (D+BV	increased $f_{\rm b}$ and reduced	$f_{\rm b}$ control, breaths/min	Bonferroni post-hoc	30	5	8		0.0385		
restoration) on the ventilatory responses	V_T compared to control.	<i>f</i> _b D+BV restoration, breaths/min	analysis	33	3	8				
during		V_{T} control, I		2.25	0.46	8		0.0133		
prolonged		V _T D+BV		2.08	0.37	8				
exercise		restoration, I								
compared to		P _{ET} CO ₂		39	4	8		0.8357		
euhydration control?		control, mmHg								
control?		P _{ET} CO ₂ D+BV		39	3	8				
		restoration,								
29. What are	T _c exhibited	mmHg T _c , °C	Two-way	n/a	n/a	4	Time	<0.0001	Time vs 10-30 min –	Table 4
the effects of	significant	1 _c , U	ANOVA with	II/a	n/a	4	Time	<0.0001	DH (*) 40-60, 85-90 &	Table 4
adrenaline (A)	differences		repeated						120 min: <0.0001 ;	N = 7
infusion on	overtime in		measures with			2	Infusion	0.0029	0.0013; 0.0001	participants
body	the A and S,		Bonferroni							F F
temperature	but T _{sk}		post-hoc			8	Time x Infusion	0.0007	Time vs 10-30 min –	Overtime
responses	remained		analysis						Control (*) 40-60, 85-	differences
during	unchanged.								90 & 120 min: 0.0026;	were
prolonged									0.0001; 0.0002	compared
exercise	Time (min) x									to 10 min of
compared to	condition (A								A vs S control (†)	exercise.
		T _{ek} ⁰C				4	Time	0.0014		
	•	isk, C				т				
	between time									
	and					2	Infusion	0.0303		
saline (S) infusion control?	vs. S) interactions. There were significant interactions between time	T _{sk} , °C				4	Time Infusion	0.0014	40-60, 85-90 & 120 min: 0.0009; 0.0127; 0.0066 Time vs 10-30 min – DH (*) NS	

	conditions in T_c but not in T_{sk} .					8	Time x Infusion	0.8743	Time vs 10-30 min – Control (*) NS A vs S control (†) NS	
30. What are	VO ₂ and	VO ₂ , I/min	Two-way	n/a	n/a	4	Time	0.0003	Time vs 10-30 min –	Table 4
the effects of	VCO ₂		ANOVA with			2	Infusion	0.1194	A (*) 40-60, 85-90 &	
adrenaline (A) infusion on	exhibited significant		repeated measures with			8	Time x Infusion	0.1031	120 min: 0.0154; 0.040; 0.0392	N = 7 participants
VO ₂ and VCO ₂ responses during prolonged exercise compared to saline (S) infusion	differences overtime in the A and S. Time (min) x condition (A vs. S) interactions.		Bonferroni post-hoc analysis						Time vs 10-30 min – Control (*) 40-60, 85- 90 & 120 min: 0.0180; 0.0450; 0.0499 A vs S control (†) NS	Overtime differences were compared to 10 min of exercise.
control?	There were	VCO ₂ , I/min	_			4	Time	0.0036	Time vs 10-30 min –	-
	significant interactions	_,				2	Infusion	0.0222	A (*) 40-60, 85-90 & 120 min: 0.0154;	
	between time and conditions in both VO_2 and VCO_2 .					8	Time x Infusion	0.0024	0.040; 0.0392 Time vs 10-30 min – Control (*) NS	
									A vs S control (†) 40-60, 85-90 & 120 min: <0.0001; 0.0105; 0.0196	
31. What are the effects of adrenaline (A)	P _{ET} CO ₂ exhibited significant	P _{ET} CO ₂ , mmHg	Two-way ANOVA with repeated	n/a	n/a	4	Time	0.0301	Time vs 10-30 min – A (*) 40-60 & 85-90 min: 0.0011; 0.0044	Table 4 N = 7
infusion on end-tidal PCO ₂ responses during	differences overtime in the A and S.		measures with Bonferroni post-hoc analysis			2	Infusion	0.4991	Time vs 10-30 min – Control (*) NS	participants Overtime differences

prolonged exercise compared to saline (S) infusion control?	Time (min) x condition (A vs. S) interactions. There were significant interactions between time and conditions in $P_{ET}CO_{2.}$					8	Time x Infusion	0.0288	A vs S control (†) 40-60 min: <0.0063	were compared to 10 min of exercise.
32. What are	Plasma	[NA], mmol/l	Two-way	n/a	n/a	4	Time	0.0069	Time vs 10-30 min –	Table 4
the effects of adrenaline (A)	catecholamin es exhibited		ANOVA with repeated			2	Infusion	0.6384	A (*) 85-90 & 120 min: 0.0390; 0.0422	N = 7
infusion on	significant		measures with			8	Time x Infusion	0.4303	- 0.0030, 0.0422	participants
circulating catecholamine	differences overtime in		Bonferroni post-hoc						Time vs 10-30 min – Control (*) NS	Overtime
s responses during prolonged	the A and to lesser extend in S.		analysis						A vs S control (†) NS	differences were compared
exercise compared to saline (S)	Time (min) x condition (A	[A], mmol/l				4	Time	<0.0001	Time vs 10-30 min – A (*) 40-60, 85-90 & 120 min: 0.0081;	to 10 min of exercise.
infusion control?	vs. S) interactions.					2	Infusion	0.0025	0.0120; 0.0373	
	There were significant interactions between time					8	Time x Infusion	<0.0001	Time vs 10-30 min – Control (*)120 min: 0.0222	
	and conditions in [A] but not in [NA].								A vs S control (†) 40-60; 85-90 & 120 min: 0.0013; 0.0020; 0.0130	