SUPPLEMENTARY MATERIAL

Home-cage gastrocnemius and BAT temperatures after intra-VMH MTII in HCR and LCR.

Hind limb muscle and BAT temperatures were measured at baseline and every 15 min for 240 min after intra-VMH vehicle or MTII microinjection in HCR and LCR. Changes in temperature from individual baseline values were calculated to factor out individual differences in baseline temperature. Intra-VMH MTII induced some change in temperature in BAT in HCR and LCR rats (Figure S1), but was less effective in inducing changes in muscle temperature (Figure S2).

For BAT, there was a small increase in temperature which peaked about one hour after injection. There was a main effect of time but not MTII on BAT temperature, and a significant interaction where the effect of MTII depended on the time after injection. Because of the significant interaction between line and time, HCR and LCR were analyzed separately; in both lines, there was a main effect of MTII and an interaction between MTII and time, where the MTII-induced BAT thermogenesis depended on time since treatment.

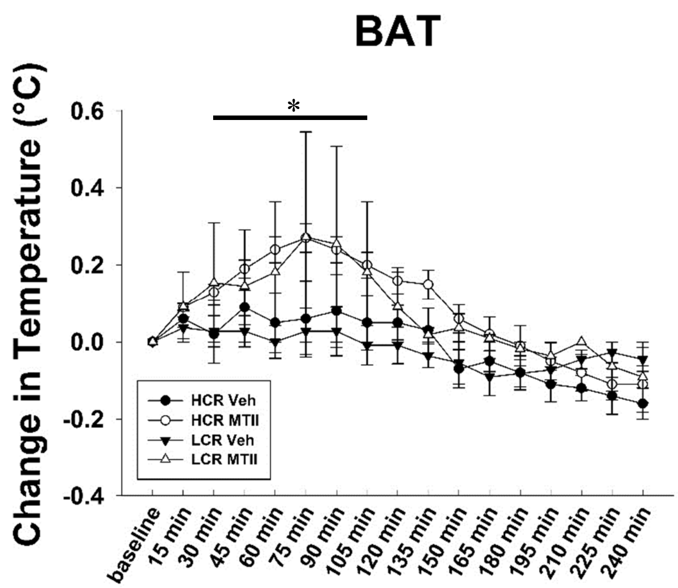
There was a significant interaction in change in BAT temperature from baseline where MTII induced a significant deviation from baseline temperature but vehicle microinjection did not. There were also significant main effects of time and MTII, and an interaction between line and time since injection (see Figure S1).

In both the left and right gastrocnemius muscles groups, there was a main effect of time where temperature changed over time. This follows the daily rhythm in baseline muscle temperature we have demonstrated previously (where temperature falls throughout the light phase), which in turn follows the daily rhythm in physical activity levels. There were no main effects of line

(HCR/LCR) or MTII in either the right or left gastrocnemius temperatures. The right gastrocnemius showed a significant interaction where HCR and LCR showed different temperatures depending on the time after injection, but this did not interact with MTII. Similarly, for the mean temperature of both left and right gastrocnemius, there was a main effect of time where mean gastrocnemius temperature changed over time, but no other main effects or interactions.

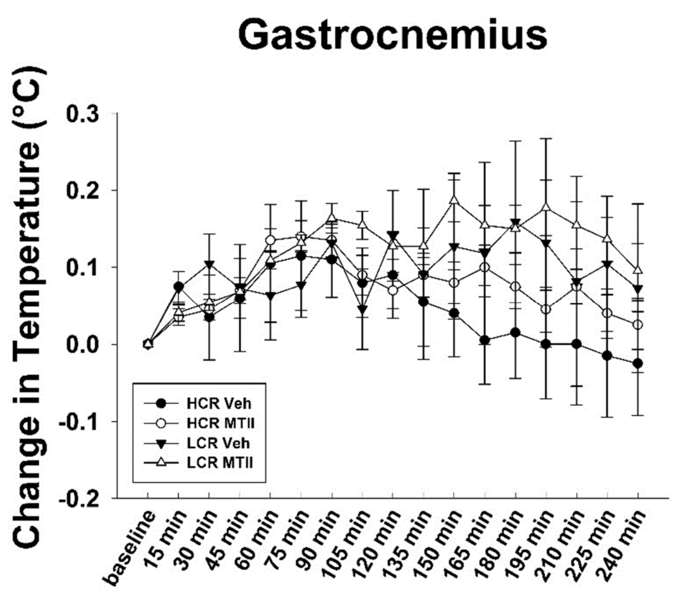
As shown in Figure S2, when change in gastrocnemius temperature was calculated according to each rat's baseline temperature, there were no significant main effects of line or MTII, but the right gastrocnemius showed a main effect of time where the change in temperature from baseline changed over time (trend in the left gastrocnemius, $p=0.057$). There was a significant MTII-by-time interaction where the right gastrocnemius showed a significant increase in temperature from baseline in the right leg in the latter half of the test, but this did not differ between HCR and LCR. Similarly, for the mean temperature of both left and right gastrocnemius, there was a main effect of time where change in temperature from baseline changed over time, but no other main effects or interactions.

Figure S1.



Brown adipose tissue (BAT) change from baseline temperature in high- and low-capacity runners (HCR, LCR) after intra-ventromedial hypothalamic microinjection of vehicle (Veh) or the mixed melanocortin receptor agonist Melanotan II (MTII). Compared to vehicle microinjection, MTII induced a significantly greater increase in temperature above baseline temperature in both HCR (45 min-105 min, and at 150 min after MTII) and LCR (15 min-105 min, and at 165 min after MTII; *$p<0.05$).

Figure S2.



Mean right and left gastrocnemius temperature over 4 hours in the home cage, increase above baseline temperature in high- and low-capacity runners (HCR, LCR) after intra-ventromedial hypothalamic microinjection of vehicle (Veh) or the mixed melanocortin receptor agonist Melanotan II (MTII). The increase in temperature above baseline levels changed over time, but there were not differences between HCR and LCR, and no significant effect of MTII compared to vehicle treatment.

Methods

mRNA and protein expression

Following assay IDs were obtained from IDT technologies for gene expression assays – Gapdh, Rn.PT. 39a.11180736.g; Beta3 adrenergic receptor, Rn.PT.58.35740415; UCP1, Rn.PT.56a.14277400; PPARα, Rn.PT.58.35766078; PPARδ, Rn.PT.58.6572075; PPARγ, Rn.PT.58.6036576; PGC1α, Rn.PT.58.37655048; UCP2, Rn.PT.58.12555837; UCP3, Rn.PT.58.17938212; SERCA1, Rn.PT.58.35312973; SERCA2, Rn.PT.58.8873034; Kir6.1, Rn.PT.58.38199111; Med1, Rn.PT.58.8279221. Probes were diluted as per IDT instructions before proceeding to quantification of gene expression. Data were calculated using ΔCt method and all data are expressed using mean ± SEM relative to HCR vehicle group set at 100%

To evaluate protein expression, primary antibodies against beta 3 adrenergic receptor, UCP1, PPARα, PPARδ, PPARγ, PGC1α, ACC, p-ACC, AMPK, p-AMPK, CD36, FAS, UCP2, beta2 adrenergic receptor, UCP3, SERCA1, SERCA2 (ab101095, ab10983, ab8934, ab23673, ab41928, ab54481, ab45174, ab68191, ab80039, ab133448, ab64014, ab22759, ab67241, ab182136, ab3477, ab2819, ab2861 respectively from Abcam); Kir6.2 and MED1 (sc-11226 and sc-5334 from Santa Cruz), and Kir6.1 (SAB2101220, Sigma-Aldrich) were obtained and incubated with the blot overnight at 4°C and with either anti-rabbit or anti-mouse secondary (ab6721, ab6789 respectively from Abcam) for 1 hr at room temperature. Blots were developed using an Amersham chemiluminescence kit and data expressed as mean ± SEM relative to HCR vehicle group set at 100%.

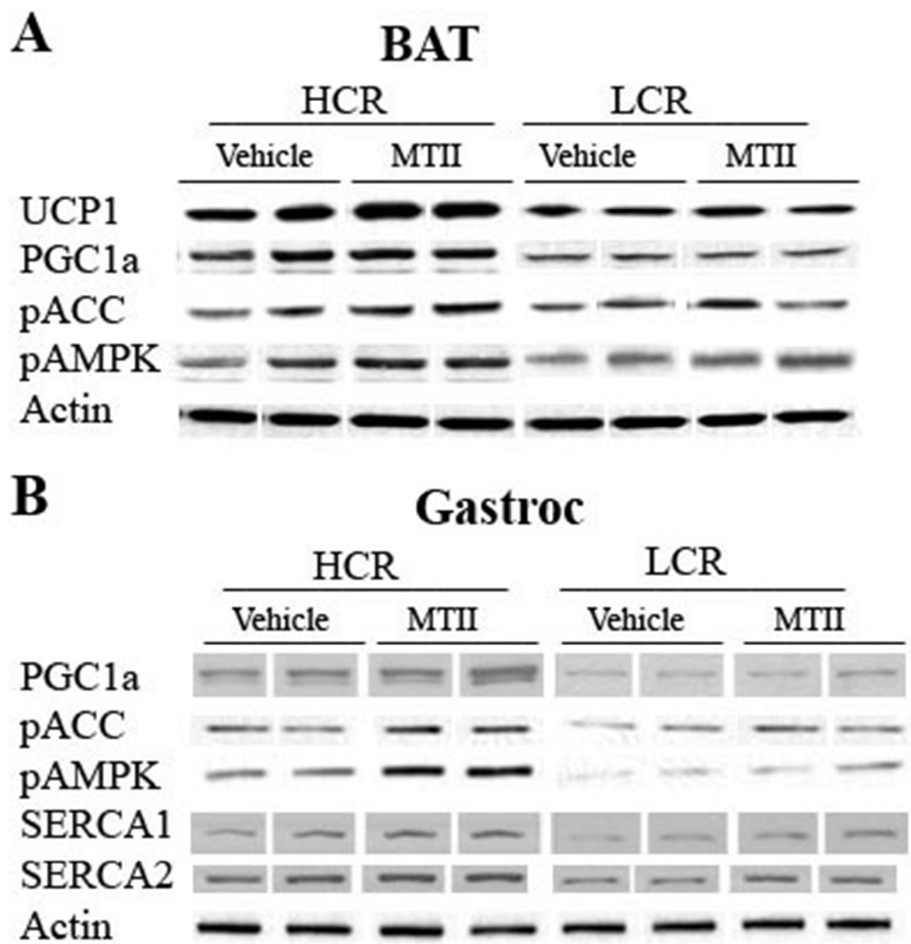


Figure S3. Representative Western blot images of (A) brown adipose tissue (BAT) and (B) gastrocnemius (gastroc) muscle of HCR and LCR treated with either the non-specific melanocortin receptor agonist Melanotan II (MTII) or vehicle (aCSF) in the ventromedial hypothalamus.

Table S1. Body weight and composition in high- and low-capacity runners (HCR, LCR) treated with vehicle (veh) and melanotan II (MTII); Mean±SEM

		HCR			LCR		
Experiment		**vehicle**	**MTII**	percent change	**vehicle**	**MTII**	percent change
Home-cage gastrocnemius & BAT temperature	BW	413.31 ±18.75	410.15 ±18.86	veh>MTII 0.77%	500.11 ±21.15	496.01 ±20.85	veh>MTII 0.83%
4-hr energy expenditure	BW	407.92 ±19.21	410.60 ±18.89		494.13 ±19.79	496.27 ±20.43	
	fat mass	66.99 ±7.49	67.3 ±7.50		106.76 ±11.70	107.5 ±11.49	
	lean mass	252.26 ±10.57	255.44 ±10.70	MTII>veh 1.26%	288.51 ±11.05	286.50 ±11.24	
Treadmill activity thermogenesis	BW	405.63 ±19.28	408.33 ±19.41	MTII>veh 0.67%	487.53 ±26.54	490.19 ±27.92	
	fat mass	68.29 ±7.20	68.76 ±7.22	MTII>veh 0.69%	106.73 ±15.74	107.43 ±16.16	
	lean mass	255.20 ±11.62	256.8 ±11.69	MTII>veh 0.66%	285.3 ±8.62	286.78 ±9.33	
Treadmill activity energy expenditure	BW	401.00 ±18.59	403.64 ±19.52		505.55 ±21.12	500.29 ±20.90	veh>MTII 1.05%
	fat mass	59.48 ±6.84	60.00 ±7.40		114.65 ±13.18	110.27 ±12.48	
	lean mass	251.20 ±10.24	260.61 ±11.51		293.62 ±11.63	283.88 ±10.65	veh>MTII 3.43%

Percent change reported on values that showed significant change between treatments, within line ($p<0.05$). Body weights taken immediately before microinjection; lean and fat mass measured 2 days prior to microinjection. BAT, brown adipose tissue; BW, body weight.

Table S2. Statistical results from analyses examining the effect of intra-ventromedial hypothalamic (VMH) Melanotan II (MTII) and vehicle on gas-exchange variables and physical activity in high- and low-capacity runners (HCR, LCR).

Home-cage energy expenditure			Main effects		Interaction
			MTII/vehicle	**HCR/LCR**	
	VO_2 (ml/kg/hr)	F	314.85	26.925	20.585
		df	1,19	1,19	1,19
		p	<0.001	<0.001	<0.001
	VCO_2 (ml/kg/hr)	F	244.741	28.812	22.3
		df	1,19	1,19	1,19
		p	<0.001	<0.001	<0.001
	RER	F	43.193	0.031	0.053
		df	1,19	1,19	1,19
		p	<0.001	0.863	0.820
	EE (kcal/hr)	F	323.878	0.668	9.415
		df	1,19	1,19	1,19
		p	<0.001	0.424	0.006
	Horizontal activity counts	F	92.459	9.563	12.292
		df	1,19	1,19	1,19
		p	<0.001	0.006	0.049524
	Ambulatory activity counts	F	63.003	4.263	0.536
		df	1,19	1,19	1,19
		p	<0.001	0.048	0.473
	Vertical activity counts	F	1.145	11.927	0.542
		df	1,19	1,19	1,19
		p	0.707	0.003	0.471
Analysis of covariance					
	EE with body weight as covariate	F	0.224	9.694	15.062
		df	1,17	1,17	1,17
		p	0.642	0.006	0.001
	EE with lean mass as covariate	F	0.657	12.406	8.474
		df	1,17	1,17	1,17
		p	0.429	0.003	0.010
Each covariate was significant, and there were no interactions between treatment (effect of MTII) and covariates					

EE, energy expenditure; RER, respiratory exchange ratio (VCO_2/VO_2).

Table S3. Statistical results from analyses examining the effect of intra-ventromedial hypothalamic (VMH) Melanotan II (MTII) and vehicle on gas-exchange variables in high- and low-capacity runners (HCR, LCR) during treadmill walking activity (walking 7 meters/min for 30 min).

Treadmill energy expenditure			Main effects		Interaction
			MTII/vehicle	**HCR/LCR**	
	VO_2 (ml/kg/hr)	F	27.266	1.604	3.473
		df	1,14	1,14	1,14
		p	<0.001	0.226	0.084
	VCO_2 (ml/kg/hr)	F	0.976	0.081	26.192
		df	1,14	1,14	1,14
		p	0.34	0.781	<0.001
	RER	F	57.619	23.059	5.461
		df	1,14	1,14	1,14
		p	<0.001	<0.001	0.035
	EE (kcal/hr)	F	38.207	7.136	11.114
		df	1,14	1,14	1,14
		p	<0.001	0.018	0.005
Analysis of covariance					
	EE with body weight as covariate	F	0.079	0.198	8.555
		df	1,13	1,13	1,13
		p	0.783	0.664	0.012
	EE with lean mass as covariate	F	0.15	2.342	8.946
		df	1,13	1,13	1,13
		p	0.705	0.15	0.100
Each covariate was significant, and there were no interactions between treatment (effect of MTII) and covariates					

EE, energy expenditure; RER, respiratory exchange ratio (VCO_2/VO_2).

Table S4. Statistical results from analyses examining the effect of intra-ventromedial hypothalamic (VMH) Melanotan II (MTII) and vehicle on gastrocnemius muscle temperature over the course of 4 hrs after treatment in high- and low-capacity runners (HCR, LCR).

Home cage muscle temperature		Main effects			Interactions			
		MTII/vehicle	**Time**	**HCR/LCR**	**Treatment x line**	**Time x line**	**Treatment x time**	**Treatment x time x line**
Right leg temperature	F	0.081	4.330	1.264	0.183	1.682	0.541	0.411
	df	1,19	16,4	1,19	1,19	16,4	16,4	16,4
	p	0.779	<0.001	0.275	0.673	0.049	0.924	0.979
Left leg temperature	F	1.170	1.826	0.693	0.001	1.289	0.924	0.533
	df	1,19	16,4	1,19	1,19	16,4	16,4	16,4
	p	0.393	0.027	0.415	0.975	0.202	0.542	0.929
Average L and R leg temperature	F	0.129	2.622	1.001	0.066	1.610	0.760	0.296
	df	1,19	16,4	1,19	1,19	16,4	16,4	16,4
	p	0.724	0.001	0.330	0.801	0.065	0.730	0.997
Right leg temperature change from baseline	F	2.973	2.939	0.842	0.295	1.354	2.454	0.436
	df	1,19	16,4	1,19	1,19	16,4	16,4	16,4
	p	0.101	<0.001	0.370	0.593	0.164	0.002	0.972
Left leg temperature change from baseline	F	0.091	1.644	1.138	0.338	1.655	0.633	0.359
	df	1,19	16,4	1,19	1,19	16,4	16,4	16,4
	p	0.766	0.057	0.229	0.568	0.055	0.856	0.990
Average R and L leg temperature change from baseline	F	0.550	2.622	1.183	0.003	1.610	0.760	0.296
	df	1,19	16,4	1,19	1,19	16,4	16,4	16,4
	p	0.467	0.001	0.290	0.957	0.065	0.730	0.997
BAT temperature	F	0.260	29.481	0.042	0.372	2.471	5.363	0.503
	df	1,19	16,4	1,19	1,19	16,4	16,4	16,4
	p	0.616	<0.001	0.841	0.549	0.002	p<0.001	0.945
BAT temperature change from baseline	F	8.816	29.481	0.072	0.005	2.471	5.363	0.503
	df	1,19	16,4	1,19	1,19	16,4	16,4	16,4
	p	0.008	<0.001	0.792	0.945	0.002	<0.01	0.945

Table S5. Statistical results from analyses examining the effect of intra-ventromedial hypothalamic (VMH) Melanotan II (MTII) and vehicle on gastrocnemius muscle temperature during treadmill walking in high- and low-capacity runners (HCR, LCR).

Treadmill-activity muscle temperature		Main effects			Interactions			
		MTII/vehicle	**Time**	**HCR/LCR**	**Treatment x line**	**Time x line**	**Treatment x time**	**Treatment x time x line**
Right leg temperature	F	1.470	127.156	0.016	0.007	20.176	3.773	3.524
	df	1,14	5,10	1,14	1,14	5,10	5,10	5,10
	p	0.245	<0.001	0.900	0.933	<0.001	0.004	0.007
Left leg temperature	F	0.224	199.702	0.091	0.001	19.409	3.161	2.534
	df	1,14	5,10	1,14	1,14	5,10	5,10	5,10
	p	0.643	<0.001	0.767	0.971	<0.001	0.120	0.036
Average L and R leg temperature	F	0.860	190.009	0.006	0.005	24.529	4.772	4.219
	df	1,14	5,10	1,14	1,14	5,10	5,10	5,10
	p	0.370	<0.001	0.937	0.947	<0.001	0.001	0.002
Right leg temperature change from baseline	F	0.878	127.165	16.039	0.878	20.176	3.773	3.524
	df	1,14	5,10	1,14	1,14	5,10	5,10	5,10
	p	0.365	<0.001	0.001	0.365	<0.001	0.004	0.007
Left leg temperature change from baseline	F	2.754	199.702	17.345	0.172	19.409	3.161	2.534
	df	1,14	5,10	1,14	1,14	5,10	5,10	5,10
	p	0.119	<0.001	0.001	0.685	<0.001	0.120	0.036
Average R and L leg temperature change from baseline	F	2.577	199.009	18.920	0.706	24.529	4.772	4.219
	df	1,14	5,10	1,14	1,14	5,10	5,10	5,10
	p	0.131	<0.001	0.001	0.415	<0.001	0.001	0.002
BAT temperature (before and after activity)	F	1.170	11.051	1.061	1.453	10.000	2.133	0.050
	df	1,14	1,14	1,14	1,14	1,14	1,14	1,14
	p	0.231	<0.001	0.320	0.248	0.007	0.166	0.825
BAT temperature change from baseline	F	2.133		10.000	0.050			
	df	1,14	N/A	1,14	1,14	N/A	N/A	N/A
	p	0.166		0.007	0.825			

Analysis included temperatures though 20 min of treadmill walking to encompass data for all rats, before any rats became noncompliant with treadmill-waling protocol. BAT temperatures were measured once before and once after treadmill walking (significant decrease over time, larger decrease in HCR).

Norepinephrine turnover (NETO)		Main effects		Interaction
		MTII/vehicle	**HCR/LCR**	
BAT	F	572.245	52.627	127.835
	df	1,13	1,13	1,13
	p	<0.001	<0.001	<0.001
MWAT	F	115.306	21.661	27.274
	df	1,13	1,13	1,13
	p	<0.001	<0.001	<0.001
RWAT	F	160.033	1.961	1.116
	df	1,13	1,13	1,13
	p	<0.001	0.183	0.309
EWAT	F	309.205	11.503	11.883
	df	1,13	1,13	1,13
	p	<0.001	0.004	0.004
GWAT	F	314.589	13.91	202.977
	df	1,13	1,13	1,13
	p	<0.001	0.002	<0.001
IWAT	F	258.613	8.474	65.392
	df	1,13	1,13	1,13
	p	<0.001	0.012	<0.001
Liver	F	107.912	7.332	19.324
	df	1,13	1,13	1,13
	p	<0.001	0.018	0.001
Heart	F	166.185	3.935	4.952
	df	1,13	1,13	1,13
	p	<0.001	0.069	0.044
Soleus	F	276.059	70.096	46.498
	df	1,13	1,13	1,13
	p	<0.001	<0.001	<0.001
EDL	F	392.559	9.193	6.119
	df	1,13	1,13	1,13
	p	<0.001	0.009	0.027
Quadriceps	F	298.337	36.944	9.053
	df	1,13	1,13	1,13
	p	<0.001	<0.001	0.009
Lateral gastrocnemius	F	290.798	17.969	10.142
	df	1,13	1,13	1,13
	p	<0.001	0.001	0.007
Medial gastrocnemius	F	193.457	5.539	2.903
	df	1,13	1,13	1,13
	p	<0.001	0.034	0.110

Table S6. Statistical results from analyses examining the effect of intra-ventromedial hypothalamic (VMH) Melanotan II (MTII) and vehicle on norepinephrine turnover (NETO) in high- and low-capacity runners (HCR, LCR.

BAT, brown adipose tissue; MWAT, mesenteric white adipose tissue; RWAT, retroperitoneal white adipose tissue; EWAT, epididymal white adipose tissue; GWAT, gluteal white adipose tissue; IWAT, inguinal white adipose tissue; EDL, extensor digitorum longus.

Table S7. Statistical results from analyses examining the effect of intra-ventromedial hypothalamic (VMH) Melanotan II (MTII) and vehicle on brown adipose tissue (BAT) mRNA expression using qPCR in high- and low-capacity runners (HCR, LCR).

Brown adipose tissue (BAT)		Main effects		Interaction	t-test for MTII≠vehicle	
		MTII/vehicle	**HCR/LCR**		**HCR**	**LCR**
β3-AR	F	0.376	3.527	0.272		
	df	1,28	1,28	1,28		
	p	0.544	0.071	0.606	0.480	0.102
UCP1	F	19.477	60.218	1.437		
	df	1,28	1,28	1,28		
	p	<0.001	<0.001	0.241	0.001	0.020
PPARα	F	19.502	37.072	1.892		
	df	1,28	1,28	1,28		
	p	<0.001	<0.001	0.18	0.002	0.008
PPARδ	F	31.913	37.072	0.112		
	df	1,28	1,28	1,28		
	p	<0.001	<0.001	0.74	0.000	0.024
PPARγ	F	23.261	46.191	1.736		
	df	1,28	1,28	1,28		
	p	<0.001	<0.001	0.198	0.000	0.033
PGC1α	F	21.241	37.49	2.907		
	df	1,28	1,28	1,28		
	p	<0.001	<0.001	0.099	0.001	0.007

β3-AR, Beta-3 adrenergic receptor; UCP1, uncoupling protein 1; PPAR, peroxisome proliferator activated receptor; PGC1α, PPARγ coactivator-1α.

Table S8. Statistical results from analyses examining the effect of intra-ventromedial hypothalamic (VMH) Melanotan II (MTII) and vehicle on white adipose tissue (WAT) mRNA expression using qPCR in high- and low-capacity runners (HCR, LCR).

White adipose tissue (WAT)		Main effects		Interaction	t-test for MTII≠vehicle	
		MTII/vehicle	HCR/LCR		HCR	LCR
β3-AR	F	4.877	22.009	0.375		
	df	1,28	1,28	1,28		
	p	0.36	<0.001	0.545	0.050	0.101
UCP2	F	2.184	37.707	0.168		
	df	1,28	1,28	1,28		
	p	0.151	<0.001	0.685	0.129	0.194
PPARα	F	22.384	37.856	0.536		
	df	1,28	1,28	1,28		
	p	<0.001	<0.001	0.47	0.002	0.003
PPARδ	F	31.913	37.072	0.112		
	df	1,28	1,28	1,28		
	p	<0.001	<0.001	0.74	0.001	0.000
PPARγ	F	14.813	31.963	1.163		
	df	1,28	1,28	1,28		
	p	0.001	<0.001	0.29	0.005	0.012
PGC1α	F	2.235	31.472	0.473		
	df	1,28	1,28	1,28		
	p	0.146	<0.001	0.497	0.066	0.295

β3-AR, Beta-3 adrenergic receptor; UCP2, uncoupling protein 2; PPAR, peroxisome proliferator activated receptor; PGC1α, PPARγ coactivator-1α.

Table S9. Statistical results from analyses examining the effect of intra-ventromedial hypothalamic (VMH) Melanotan II (MTII) and vehicle on liver mRNA expression using qPCR in high- and low-capacity runners (HCR, LCR).

Liver		Main effects		Interaction	t-test for MTII≠vehicle	
		MTII/vehicle	**HCR/LCR**		**HCR**	**LCR**
β2-AR	F	8.913	13.8	0.056		
	df	1,28	1,28	1,28		
	p	0.006	0.001	0.814	0.042	0.016
UCP2	F	14.544	20.254	1.239		
	df	1,28	1,28	1,28		
	p	0.001	<0.001	0.275	0.002	0.033
PPARα	F	31.701	18.427	1.608		
	df	1,28	1,28	1,28		
	p	<0.001	<0.001	0.215	0.001	0.000
PPARδ	F	74.835	48.171	6.878		
	df	1,28	1,28	1,28		
	p	<0.001	<0.001	0.014	0.000	0.000
PPARγ	F	31.376	15.815	1.938		
	df	1,28	1,28	1,28		
	p	<0.001	<0.001	0.175	0.001	0.001
PGC1α	F	52.246	28.808	1.814		
	df	1,28	1,28	1,28		
	p	<0.001	<0.001	0.189	0.000	0.000

B2-AR, Beta-2 adrenergic receptor; UCP2, uncoupling protein 2; PPAR, peroxisome proliferator activated receptor; PGC1α, PPARγ coactivator-1α.

Table S10. Statistical results from analyses examining the effect of intra-ventromedial hypothalamic (VMH) Melanotan II (MTII) and vehicle on gastrocnemius mRNA expression using qPCR in high- and low-capacity runners (HCR, LCR).

Gastrocnemius		Main effects		Interaction	t-test for MTII≠vehicle	
		MTII/vehicle	HCR/LCR		HCR	LCR
β2-AR	F	9.498	22.179	2.201		
	df	1,28	1,28	1,28		
	p	0.005	<0.001	0.149	0.009	0.069
UCP2	F	13.382	19.927	0.388		
	df	1,28	1,28	1,28		
	p	0.001	<0.001	0.536	0.014	0.003
UCP3	F	23.574	15.735	0.293		
	df	1,28	1,28	1,28		
	p	<0.001	<0.001	0.592	0.002	0.002
PPARα	F	14.728	17.598	0.31		
	df	1,28	1,28	1,28		
	p	0.001	<0.001	0.582	0.003	0.020
PPARδ	F	13.702	23.685	1.572		
	df	1,28	1,28	1,28		
	p	0.001	<0.001	0.22	0.004	0.030
PPARγ	F	22.747	15.151	1.714		
	df	1,28	1,28	1,28		
	p	<0.001	0.001	0.201	0.001	0.003
PGC1α	F	9.778	13.544	0.474		
	df	1,28	1,28	1,28		
	p	0.004	0.001	0.497	0.016	0.030
SERCA1	F	11.077	25.884	2.067		
	df	1,28	1,28	1,28		
	p	<0.001	<0.001	0.162	0.003	0.095
SERCA2	F	19.816	15.613	1.93		
	df	1,28	1,28	1,28		
	p	<0.001	<0.001	0.176	0.003	0.001
Kir6.1	F	3.269	38.247	0.484		
	df	1,28	1,28	1,28		
	p	0.081	<0.001	0.492	0.059	0.206
Kir6.2	F	9.39	42.931	1.001		
	df	1,28	1,28	1,28		
	p	0.005	<0.001	0.326	0.003	0.102
MED1	F	2.071	18.853	0.268		
	df	1,28	1,28	1,28		
	p	0.161	<0.001	0.609	0.118	0.233

Muscle abbreviations (Tables S10-S11):

B2-AR, Beta-2 adrenergic receptor; UCP2 and 3, uncoupling protein 2 and 3; PPAR, peroxisome proliferator activated receptor; PGC1α, PPARγ coactivator-1α; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; Kir6.1 and 6.2, components of ATP-gated K^{+}-channel; MED1, Mediator of RNA polymerase II transcription subunit 1.

Table S11. Statistical results from analyses examining the effect of intra-ventromedial hypothalamic (VMH) Melanotan II (MTII) and vehicle on quadriceps mRNA expression using qPCR in high- and low-capacity runners (HCR, LCR).

Quadriceps		Main effects		Interaction	t-test for MTII≠vehicle	
		MTII/vehicle	**HCR/LCR**		**HCR**	**LCR**
β2-AR	F	4.352	20.736	0.269		
	df	1,28	1,28	1,28		
	p	0.046	<0.001	0.608	0.056	0.121
UCP2	F	15.45	18.064	1.042		
	df	1,28	1,28	1,28		
	p	0.001	<0.001	0.316	0.007	0.004
UCP3	F	18.581	27.072	1.723		
	df	1,28	1,28	1,28		
	p	<0.001	<0.001	0.2	0.004	0.002
PPARα	F	7.746	32.282	0.855		
	df	1,28	1,28	1,28		
	p	0.01	<0.001	0.363	0.006	0.121
PPARδ	F	7.163	35.755	3.233		
	df	1,28	1,28	1,28		
	p	0.012	<0.001	0.083	0.006	0.253
PPARγ	F	3.829	31.905	1.311		
	df	1,28	1,28	1,28		
	p	0.06	<0.001	0.262	0.034	0.261
PGC1α	F	3.86	10.493	0.926		
	df	1,28	1,28	1,28		
	p	0.059	0.003	0.344	0.033	0.237
SERCA1	F	5.653	24.607	2.303		
	df	1,28	1,28	1,28		
	p	0.024	<0.001	0.14	0.010	0.266
SERCA2	F	4.886	57.654	1.1		
	df	1,28	1,28	1,28		
	p	0.035	<0.001	0.303	0.005	0.250
Kir6.1	F	3.206	31.955	0.017		
	df	1,28	1,28	1,28		
	p	0.084	<0.001	0.897	0.069	0.156
Kir6.2	F	1.401	16.331	0.046		
	df	1,28	1,28	1,28		
	p	0.246	<0.001	0.832	0.273	0.139
MED1	F	1.304	16.024	0.084		
	df	1,28	1,28	1,28		
	p	0.263	<0.001	0.774	0.261	0.182

Table S12. Statistical results from analyses examining the effect of intra-ventromedial hypothalamic (VMH) Melanotan II (MTII) and vehicle on brown adipose tissue (BAT) protein expression using Western blot in high- and low-capacity runners (HCR, LCR).

Brown adipose tissue (BAT)		Main effects		Interaction	t-test for MTII≠vehicle	
		MTII/vehicle	HCR/LCR		HCR	LCR
β3-AR	F	2.644	11.725	0.192		
	df	1,10	1,10	1,10		
	p	0.135	0.007	0.671	0.258	0.054
UCP1	F	9.374	62.4	0.914		
	df	1,10	1,10	1,10		
	p	0.012	<0.001	0.362	0.009	0.125
PPARα	F	2.02	3.82	0.202		
	df	1,10	1,10	1,10		
	p	0.186	0.079	0.663	0.140	0.240
PPARδ	F	1.029	1.575	0.05		
	df	1,10	1,10	1,10		
	p	0.334	0.238	0.827	0.203	0.306
PPARγ	F	1.405	9.053	0.037		
	df	1,10	1,10	1,10		
	p	0.263	0.013	0.851	0.244	0.200
PGC1α	F	5.018	18.86	1.036		
	df	1,10	1,10	1,10		
	p	0.049	0.001	0.333	0.023	0.236
ACC	F	0.000	0.416	0.005		
	df	1,10	1,10	1,10		
	p	0.983	0.533	0.945	0.485	0.477
pACC	F	10.578	10.765	2.114		
	df	1,10	1,10	1,10		
	p	0.009	0.008	0.176	0.013	0.115
AMPK	F	0.039	0.053	0.000		
	df	1,10	1,10	1,10		
	p	0.847	0.822	0.996	0.438	0.453
pAMPK	F	14.061	16.655	1.558		
	df	1,10	1,10	1,10		
	p	0.004	0.002	0.24	0.008	0.069
CD36 (FAT)	F	0.24	1.324	0.016		
	df	1,10	1,10	1,10		
	p	0.635	0.277	0.903	0.412	0.323
FAS	F	0.037	0.02	0.000		
	df	1,10	1,10	1,10		
	p	0.852	0.89	0.992	0.447	0.450

Table S12 abbreviations:

B3-AR, Beta-3 adrenergic receptor; UCP1, uncoupling protein 1; PPAR, peroxisome proliferator activated receptor; PGC1α, PPARγ coactivator-1α; (p)ACC, (phosphor-)acetyl-CoA carboxylase; (p)AMPK, (phospho-)AMP-activated protein kinase; CD36, fatty acid translocase; FAS, fatty acid synthase.

Table S13. Statistical results from analyses examining the effect of intra-ventromedial hypothalamic (VMH) Melanotan II (MTII) and vehicle on white adipose tissue (WAT) protein expression using Western blot in high- and low-capacity runners (HCR, LCR).

White adipose tissue (WAT)		Main effects		Interaction	t-test for MTII≠vehicle	
		MTII/vehicle	HCR/LCR		HCR	LCR
β3-AR	F	1.87	15.865	0.089		
	df	1,10	1,10	1,10		
	p	0.201	0.003	0.772	0.135	0.251
UCP2	F	0.852	5.761	0.052		
	df	1,10	1,10	1,10		
	p	0.378	0.037	0.824	0.239	0.311
PPARα	F	1.446	1.677	0.000		
	df	1,10	1,10	1,10		
	p	0.257	0.224	0.996	0.151	0.256
PPARδ	F	0.127	0.056	0.001		
	df	1,10	1,10	1,10		
	p	0.729	0.817	0.971	0.408	0.400
PPARγ	F	2.453	2.831	0.11		
	df	1,10	1,10	1,10		
	p	0.148	0.123	0.747	0.098	0.230
PGC1α	F	2.448	6.458	0.108		
	df	1,10	1,10	1,10		
	p	0.149	0.029	0.749	0.105	0.225
ACC	F	0.003	0.479	0.000		
	df	1,10	1,10	1,10		
	p	0.959	0.505	1.000	0.486	0.486
pACC	F	4.04	1.222	0.000		
	df	1,10	1,10	1,10		
	p	0.072	0.297	0.988	0.110	0.105
AMPK	F	0.012	0.007	0.003		
	df	1,10	1,10	1,10		
	p	0.916	0.936	0.956	0.487	0.454
pAMPK	F	7.565	1.962	1.301		
	df	1,10	1,10	1,10		
	p	0.02	0.192	0.281	0.023	0.144
CD36 (FAT)	F	0.003	0.543	0.000		
	df	1,10	1,10	1,10		
	p	0.954	0.478	0.997	0.484	0.484
FAS	F	0.19	0.168	0.013		
	df	1,10	1,10	1,10		
	p	0.672	0.69	0.912	0.352	0.417

Table S13 abbreviations:

B3-AR, Beta-3 adrenergic receptor; UCP2, uncoupling protein 2; PPAR, peroxisome proliferator activated receptor; PGC1α, PPARγ coactivator-1α; (p)ACC, (phosphor-)acetyl-CoA carboxylase; (p)AMPK, (phospho-)AMP-activated protein kinase; CD36, fatty acid translocase; FAS, fatty acid synthase.

Table S14. Statistical results from analyses examining the effect of intra-ventromedial hypothalamic (VMH) Melanotan II (MTII) and vehicle on liver protein expression using Western blot in high- and low-capacity runners (HCR, LCR).

Liver		Main effects		Interaction	t-test for MTII≠vehicle	
		MTII/vehicle	HCR/LCR		HCR	LCR
β2-AR	F	1.715	9.737	0.131		
	df	1,10	1,10	1,10		
	p	0.22	0.011	0.725	0.283	0.123
UCP2	F	2.294	16.113	0.067		
	df	1,10	1,10	1,10		
	p	0.161	0.002	0.802	0.152	0.183
PPARα	F	3.693	4.178	0.096		
	df	1,10	1,10	1,10		
	p	0.084	0.068	0.763	0.110	0.121
PPARδ	F	4.3	11.853	0.629		
	df	1,10	1,10	1,10		
	p	0.065	0.006	0.446	0.054	0.194
PPARγ	F	3.628	17.526	0.265		
	df	1,10	1,10	1,10		
	p	0.086	0.002	0.618	0.054	0.209
PGC1α	F	0.693	10.01	0.521		
	df	1,10	1,10	1,10		
	p	0.425	0.01	0.487	0.181	0.467
AMPK	F	0.000	0.013	0.001		
	df	1,10	1,10	1,10		
	p	0.986	0.912	0.978	0.487	0.497
pAMPK	F	6.219	10.559	2.052		
	df	1,10	1,10	1,10		
	p	0.032	0.009	0.182	0.019	0.246
CD36 (FAT)	F	0.427	7.195	0.085		
	df	1,10	1,10	1,10		
	p	0.528	0.023	0.776	0.275	0.400
FAS	F	0.003	0.085	0.031		
	df	1,10	1,10	1,10		
	p	0.955	0.776	0.865	0.438	0.469

Table S14 abbreviations:

B3-AR, Beta-3 adrenergic receptor; UCP1, uncoupling protein 1; PPAR, peroxisome proliferator activated receptor; PGC1α, PPARγ coactivator-1α; (p)AMPK, (phospho-)AMP-activated protein kinase; CD36, fatty acid translocase; FAS, fatty acid synthase.

Table S15. Statistical results from analyses examining the effect of intra-ventromedial hypothalamic (VMH) Melanotan II (MTII) and vehicle on gastrocnemius protein expression using Western blot in high- and low-capacity runners (HCR, LCR).

Gastrocnemius		Main effects		Interaction	t-test for MTII≠vehicle	
		MTII/vehicle	HCR/LCR		HCR	LCR
β2-AR	F	3.07	21.328	0.347		
	df	1,10	1,10	1,10		
	p	0.11	0.001	0.569	0.073	0.232
UCP2	F	1.019	23.156	0.131		
	df	1,10	1,10	1,10		
	p	0.337	0.001	0.725	0.183	0.337
UCP3	F	1.5585	27.783	0.572		
	df	1,10	1,10	1,10		
	p	0.237	<0.001	0.467	0.112	0.365
PPARα	F	2.658	18.262	0.006		
	df	1,10	1,10	1,10		
	p	0.134	0.002	0.941	0.157	0.142
PPARδ	F	3.653	18.654	0.089		
	df	1,10	1,10	1,10		
	p	0.085	0.002	0.771	0.106	0.130
PPARγ	F	4.359	20.371	0.517		
	df	1,10	1,10	1,10		
	p	0.063	0.001	0.489	0.059	0.177
PGC1α	F	5.534	31.995	1.272		
	df	1,10	1,10	1,10		
	p	0.04	<0.001	0.286	0.023	0.226
SERCA1	F	5.843	31.356	0.581		
	df	1,10	1,10	1,10		
	p	0.036	<0.001	0.463	0.026	0.169
SERCA2	F	3.609	42.549	0.991		
	df	1,10	1,10	1,10		
	p	0.087	<0.001	0.343	0.051	0.271
Kir6.1	F	0.161	12.388	0.000		
	df	1,10	1,10	1,10		
	p	0.697	0.006	0.998	0.384	0.402
Kir6.2	F	0.057	27.232	0.037		
	df	1,10	1,10	1,10		
	p	0.817	<0.001	0.851	0.377	0.489
MED1	F	0.085	33	0.057		
	df	1,10	1,10	1,10		
	p	0.777	<0.001	0.816	0.487	0.357

Table S15-S16 abbreviations:

B2-AR, Beta-2 adrenergic receptor; UCP2 and 3, uncoupling protein 2 and 3; PPAR, peroxisome proliferator activated receptor; PGC1α, PPARγ coactivator-1α; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; Kir6.1 and 6.2, components of ATP-gated K^{+}-channel; MED1, Mediator of RNA polymerase II transcription subunit 1; (p)AMPK, (phospho-)AMP-activated protein kinase; CD36, fatty acid translocase; FAS, fatty acid synthase.

ACC	F	0.118	1.134	0.102		
	df	1,10	1,10	1,10		
	p	0.738	0.312	0.756	0.494	0.321
pACC	F	10.516	28.566	0.986		
	df	1,10	1,10	1,10		
	p	0.009	<0.001	0.344	0.015	0.086
AMPK	F	0.002	0.012	0.001		
	df	1,10	1,10	1,10		
	p	0.965	0.917	0.973	0.479	0.497
pAMPK	F	11.619	25.222	0.757		
	df	1,10	1,10	1,10		
	p	0.007	0.001	0.405	0.012	0.074
CD36 (FAT)	F	2.41	14.068	0.156		
	df	1,10	1,10	1,10		
	p	0.152	0.004	0.701	0.114	0.224
FAS	F	0.04	1.211	0.024		
	df	1,10	1,10	1,10		
	p	0.845	0.297	0.879	0.408	0.488

Table S16. Statistical results from analyses examining the effect of intra-ventromedial hypothalamic (VMH) Melanotan II (MTII) and vehicle on quadriceps protein expression using Western blot in high- and low-capacity runners (HCR, LCR).

Quadriceps		**Main effects**		**Interaction**	**t-test for MTII≠vehicle**	
		MTII/vehicle	**HCR/LCR**		**HCR**	**LCR**
β2-AR	F	0.633	7.983	0.027		
	df	1,10	1,10	1,10		
	p	0.445	0.018	0.872	0.275	0.327
UCP2	F	0.494	23.761	0.118		
	df	1,10	1,10	1,10		
	p	0.498	0.001	0.739	0.251	0.402
UCP3	F	0.766	24.987	0.263		
	df	1,10	1,10	1,10		
	p	0.402	0.001	0.619	0.198	0.398
PPARα	F	0.731	6.775	0.078		
	df	1,10	1,10	1,10		
	p	0.413	0.026	0.785	0.245	0.338
PPARδ	F	0.02	6.22	0.066		
	df	1,10	1,10	1,10		
	p	0.89	0.032	0.802	0.401	0.467
PPARγ	F	1.151	10.182	0.303		
	df	1,10	1,10	1,10		
	p	0.173	0.01	0.594	0.126	0.249

PGC1α	F	2.279	30.836	0.196		
	df	1,10	1,10	1,10		
	p	0.162	<0.001	0.667	0.109	0.246
SERCA1	F	3.919	19.141	0.397		
	df	1,10	1,10	1,10		
	p	0.076	0.001	0.543	0.047	0.212
SERCA2	F	2.538	20.539	0.13		
	df	1,10	1,10	1,10		
	p	0.142	0.001	0.726	0.116	0.208
Kir6.1	F	0.118	3.612	0.01		
	df	1,10	1,10	1,10		
	p	0.739	0.087	0.923	0.418	0.400
Kir6.2	F	0.405	24.289	0.000		
	df	1,10	1,10	1,10		
	p	0.539	0.001	0.985	0.336	0.335
MED1	F	0.216	32.532	0.024		
	df	1,10	1,10	1,10		
	p	0.652	<0.001	0.881	0.415	0.344
ACC	F	0.162	0.877	0.020		
	df	1,10	1,10	1,10		
	p	0.695	0.731	0.890	0.435	0.346
pACC	F	3.077	5.656	0.330		
	df	1,10	1,10	1,10		
	p	0.110	0.039	0.578	0.081	0.220
AMPK	F	0.010	0.000	0.000		
	df	1,10	1,10	1,10		
	p	0.923	0.991	0.995	0.472	0.474
pAMPK	F	6.679	20.554	0.300		
	df	1,10	1,10	1,10		
	p	0.027	0.001	0.596	0.041	0.101
CD36 (FAT)	F	0.081	6.323	0.039		
	df	1,10	1,10	1,10		
	p	0.782	0.031	0.847	0.369	0.477
FAS	F	0.002	0.002	0.024		
	df	1,10	1,10	1,10		
	p	0.966	0.964	0.880	0.452	0.466