Supplemental Information for

Chronic mirabegron treatment increases human brown fat, HDL cholesterol, and insulin sensitivity

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Contents: Supplemental Tables = 13, Figures = 8

Supplemental Table 1. Mirabegron Pharmacokinetic Parameters

Pharmacokinetic Measure (Units)	Day 1	Day 28	*P* Value
C_{max}[A] (nM)	184 ± 27	305 ± 45	0.02
t_{max}[B] (min)	180 (120-300)	210 (60-300)	0.94
AUC_{0-300}[C] (nM*min)	23,719 ± 3,229	45,342 ± 5,719	0.001
Trough Levels (nM)[D]			
Day 14	40.3 ± 5.0		
Day 28	45.2 ± 4.5		
16 days after cessation	5.4 ± 0.1		

[A]Mean ± SEM;
[B]Median (Range);
[C]AUC from time of oral dose administration to PET/CT scan, reported as mean ± SEM;
[D]n=14 for Days 14 and 28; n=6 for 16 days after cessation

Supplemental Table 2. Parameters of the Repeated-Measures ANOVA for REE

Summary of Fit and Parameter Estimates

RSquare Adj	0.87
Root Mean Square Error	3.22
Mean of Response	62.76

Term	Estimate	Std Error	denominator degrees of freedom	Prob>|t|
Intercept	62.76	1.92	13	<0.001
Day	-0.23	0.43	39	0.59
Time	-1.71	0.43	39	<0.001
Day*Time	-1.47	0.43	39	0.001

Least Squares Means Table for Fixed and Random Effects

Effect	Level	Least Sq Mean	Std Error	Lower 95%	Upper 95%
Day	Day 1	62.52	1.97	58.31	66.74
Day	Day 28	62.99	1.97	58.78	67.20
Time	8:00	61.04	1.97	56.83	65.26
Time	13:00	64.47	1.97	60.26	68.68
Day*Time	Day 1,08:00	59.34	2.06	54.99	63.68
Day*Time	Day 1,13:00	65.71	2.06	61.37	70.06
Day*Time	Day 28,08:00	62.75	2.06	58.41	67.10
Day*Time	Day 28,13:00	63.23	2.06	58.89	67.58
ID	1	68.26	1.57	65.09	71.43
ID	2	61.06	1.57	57.90	64.23
ID	3	56.32	1.57	53.15	59.49
ID	4	56.68	1.57	53.51	59.84
ID	5	64.23	1.57	61.06	67.40
ID	6	59.82	1.57	56.66	62.99
ID	7	68.42	1.57	65.25	71.58
ID	8	56.50	1.57	53.34	59.67
ID	9	67.86	1.57	64.69	71.03
ID	10	58.10	1.57	54.93	61.27
ID	11	65.21	1.57	62.04	68.38
ID	12	55.72	1.57	52.55	58.89
ID	13	80.16	1.57	76.99	83.33
ID	14	60.27	1.57	57.10	63.43

Least Squares Means Differences, Student's *t*

Effect	Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	P Value
Day*Time	Day 1,13:00	Day 1,08:00	6.38	1.22	3.92	8.84	<0.001
Day*Time	Day 28,13:00	Day 1,08:00	3.90	1.22	1.44	6.35	0.003
Day*Time	Day 28,08:00	Day 1,08:00	3.42	1.22	0.96	5.87	0.01
Day*Time	Day 1,13:00	Day 28,08:00	2.96	1.22	0.50	5.42	0.02
Day*Time	Day 1,13:00	Day 28,13:00	2.48	1.22	0.02	4.94	0.05
Day*Time	Day 28,13:00	Day 28,08:00	0.48	1.22	-1.98	2.94	0.70

Supplemental Table 3. Parameters of the Repeated-Measures ANOVA for RQ

Summary of Fit and Parameter Estimates

RSquare Adj	0.82
Root Mean Square Error	0.018
Mean of Response	0.809

Term	Estimate	Std Error	denominator degrees of freedom	Prob>|t|
Intercept	0.8094	0.0049	13	<0.001
Day	0.0004	0.0024	39	0.88
Time	0.0301	0.0024	39	<0.001
Day*Time	0.0046	0.0024	39	0.06

Least Squares Means Table for Fixed and Random Effects

Effect	Level	Least Sq Mean	Std Error	Lower 95%	Upper 95%
Day	Day 1	0.810	0.0054	0.798	0.821
Day	Day 28	0.809	0.0054	0.798	0.820
Time	8:00	0.840	0.0054	0.828	0.851
Time	13:00	0.779	0.0054	0.768	0.791
Day*Time	Day 1,08:00	0.845	0.0064	0.831	0.858
Day*Time	Day 1,13:00	0.775	0.0064	0.762	0.788
Day*Time	Day 28,08:00	0.835	0.0064	0.822	0.848
Day*Time	Day 28,13:00	0.784	0.0064	0.771	0.797
ID	1	0.797	0.0079	0.781	0.813
ID	2	0.799	0.0079	0.783	0.814
ID	3	0.788	0.0079	0.772	0.804
ID	4	0.811	0.0079	0.796	0.827
ID	5	0.821	0.0079	0.805	0.836
ID	6	0.821	0.0079	0.805	0.836
ID	7	0.793	0.0079	0.777	0.809
ID	8	0.831	0.0079	0.815	0.847
ID	9	0.806	0.0079	0.790	0.822
ID	10	0.799	0.0079	0.783	0.815
ID	11	0.822	0.0079	0.806	0.837
ID	12	0.806	0.0079	0.790	0.822
ID	13	0.830	0.0079	0.815	0.846
ID	14	0.809	0.0079	0.793	0.825

Least Squares Means Differences, Student's *t*

Effect	Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	P Value
Day*Time	Day 1,08:00	Day 1,13:00	0.0694	0.0068	0.0557	0.0832	<0.001
Day*Time	Day 1,08:00	Day 28,13:00	0.0609	0.0068	0.0472	0.0747	<0.001
Day*Time	Day 28,08:00	Day 1,13:00	0.0595	0.0068	0.0458	0.0732	<0.001
Day*Time	Day 28,08:00	Day 28,13:00	0.0510	0.0068	0.0373	0.0647	<0.001
Day*Time	Day 1,08:00	Day 28,08:00	0.0099	0.0068	-0.0038	0.0237	0.15
Day*Time	Day 28,13:00	Day 1,13:00	0.0085	0.0068	-0.0052	0.0222	0.22

Supplemental Table 4. Cardiovascular Parameters

	Day 1	Day 28	Change[C]
Heart Rate[A] (bpm)	08:00: 75.8 ± 2.5 13:00: 82.2 ± 2.8 +6.4 ± 2.3 *P* = 0.02	08:00: 85.3 ± 2.0 13:00: 84.8 ± 2.8 -0.6 ± 1.3 *P* = 0.68	*P* < 0.001 *P* = 0.13 *P* = 0.01
Systolic Blood Pressure[B] (mmHg)	08:00: 111.6 ± 2.3 13:00: 119.8 ± 2.7 +8.2 ± 2.5 *P* = 0.006	08:00 114.5 ± 1.9 13:00: 115.4 ± 2.9 +0.9 ± 2.4 *P* = 0.72	*P* = 0.26 *P* = 0.06 P = 0.04
Diastolic Blood Pressure[B] (mmHg)	08:00: 67.0 ± 2.2 13:00: 68.9 ± 2.2 + 1.9 ± 1.0 *P* = 0.08	08:00: 70.4 ± 2.3 13:00: 70.6 ± 1.7 +0.2 ± 1.3 *P* = 0.89	*P* = 0.06 *P* = 0.21 *P* = 0.25
Rate Pressure Product[D] (mmHg*bpm)	08:00: 8461 ± 319 13:00: 9890 ± 493 +1429 ± 441 *P* = 0.006	08:00: 9774 ± 292 13:00: 9780 ± 403 +6 ± 254 *P* = 0.98	*P* < 0.001 *P* = 0.73 *P* = 0.008

Guide			
Measurement	Difference on Day 1 08:00 and 13:00 *P*-value:	Difference on Day 28 08:00 and 13:00 *P*-value:	*P*: Difference in 08:00 values *P*: Difference in 13:00 values *P*: Difference of change Day 1 vs. change Day 28

[A]Mean measurement taken continuously over a 20-minute period
[B]Mean measurement taken in triplicate over a 20-minute period
[C]Paired Student's *t*-tests.
[D]Rate Pressure Product = (Heart Rate) * (Systolic Blood Pressure)

Supplemental Table 5. Blood Levels of Common Metabolites and Hormones

Metabolite (units)	Day 1 at 08:00[A]	Δ Day 1[B]	*P* value	Day 28 at 08:00[A]	Δ Day 28[B]	*P* value	Δ 8:00-8:00 *P* Value[C]
Glucose (mg/dL)	88 ± 2	-4 ± 2	0.06	89 ± 1	-5 ± 1	0.001	0.42
Insulin (µU/mL)	10.3 ± 0.9	-0.6 ± 0.6	0.24	11.8 ± 1.1	-3.1 ± 0.9	0.006	0.15
NEFA (mEq/L)	0.37 ± 0.02	+0.54 ± 0.04	<0.001	0.36 ± 0.03	+0.30 ± 0.04	<0.001	0.60
β-hydroxybutyrate (mM)	0.03 ± 0.01	+0.27 ± 0.07	0.002	0.04 ± 0.01	+0.08 ± 0.03	0.02	0.44
Lactate (mmol/L)	0.9 ± 0.1	+0.2 ± 0.1	0.20	0.8 ± 0.1	-0.1 ± 0.1	0.46	0.29
Pyruvate (mg/dL)	0.9 ± 0.1	-0.1 ± 0.1	0.46	0.9 ± 0.1	-0.2 ± 0.1	0.16	0.28
Total bile acids (µM)	2.99 ± 0.39	-2.13 ± 0.45	0.001	4.46 ± 0.80	-2.82 ± 0.64	0.001	0.042
FGF19 (pg/mL)	118 ± 22	-62 ± 19	0.006	119 ± 20	-45 ± 20	0.037	0.97
Norepinephrine (pg/mL)	263 ± 26	+57 ± 23	0.03	231 ± 12	+107 ± 48	0.047	0.27
Epinephrine (pg/mL)	20 ± 3	+4 ± 6	0.58	20 ± 4	+0 ± 3	0.91	0.73
Dopamine (pg/mL)	13 ± 1	-1 ± 1	0.34	13 ± 0	+2 ± 2	0.17	0.34
T3, total (ng/dL)	110.4 ± 7.3	-1.4 ± 1.2	0.25	115.4 ± 8.3	-2.8 ± 1.5	0.09	0.25
T4, free (ng/dL)	1.1 ± 0.0	+0.1 ± 0.0	0.014	1.2 ± 0.0	+0.0 ± 0.0	0.05	0.17
TSH (µIU/mL)	1.59 ± 0.24	-0.34 ± 0.11	0.009	1.98 ± 0.48	-0.58 ± 0.27	0.047	0.19
Total Protein (g/dL)	6.6 ± 0.3	+0.3 ± 0.1	0.013	6.8 ± 0.1	+0.2 ± 0.1	0.004	0.44
Creatine Kinase (U/L)	79.0 ± 18.4	-3.5 ± 4.5	0.98	78.8 ± 14.6	-3.2 ± 3.1	0.33	0.99
Growth Hormone (ng/mL)	1.67 ± 0.53	-1.05 ± 0.72	0.18	1.16 ± 0.71	-0.54 ± 0.62	0.40	0.37
Glucagon (pmol/L)	24.8 ± 2.1	-1.9 ± 3.7	0.62	29.1 ± 2.5	-8.1 ± 2.7	0.012	0.20
Cortisol (µg/dL)	13.6 ± 1.3	-6.1 ± 1.0	<0.001	13.1 ± 1.3	-6.1 ± 1.4	0.001	0.61
ACTH (pg/mL)	22.9 ± 4.9	-8.0 ± 4.4	0.09	26.1 ± 4.2	-11.0 ± 3.9	0.015	0.55
PTH (pg/mL)	36.5 ± 3.0	+2.7 ± 1.6	0.11	35.3 ± 2.9	+2.9 ± 2.1	0.20	0.55
Ghrelin (pg/mL)	234 ± 45	+4 ± 28	0.89	316 ± 55	+98 ± 105	0.37	0.07
Leptin (ng/mL)	15.1 ± 2.1	-3.0 ± 0.9	0.004	17.2 ± 2.3	-4.1 ± 0.6	<0.001	0.07
Adiponectin (µg/mL)	8.56 ± 1.16	+0.63 ± 0.23	0.015	11.56 ± 1.57	+0.25 ± 0.17	0.18	0.001
FGF21 (pg/mL)	701 ± 186	+141 ± 176	0.44	819 ± 234	-295 ± 98	0.01	0.15
PYY (pg/mL)	54.6 ± 7.1	-27.1 ± 5.2	<0.001	59.2 ± 8.9	-28.4 ± 6.8	0.001	0.58
aGLP-1 (pg/mL)	1.35 ± 0.21	-0.84 ± 0.21	0.001	1.67 ± 0.29	-0.96 ± 0.25	0.002	0.22
aGIP (pg/mL)	9.1 ± 1.7	-2.6 ± 1.7	0.16	13.9 ± 3.0	-5.8 ± 3.4	0.11	0.07
tGIP (pg/mL)	34.2 ± 4.2	-9.0 ± 2.7	0.005	44.8 ± 5.5	-17.5 ± 5.7	0.009	0.026
Total Cholesterol (mg/dL)	162.8 ± 7.5	+3.3 ± 4.3	0.46	169.4 ± 8.0	-2.4 ± 4.2	0.58	0.11
Triglycerides (mg/dL)	87.3 ± 7.3	+3.2 ± 3.9	0.43	86.0 ± 7.9	-4.4 ± 3.5	0.23	0.72
HDL-C (mg/dL)	63.6 ± 5.6	-0.3 ± 1.7	0.87	69.0 ± 5.9	-1.0 ± 1.7	0.56	0.001
ApoA1 (ug/mL)	965 ± 93	+48 ± 36	0.21	1075 ± 110	+51 ± 29	0.099	0.017
ApoE (ug/mL)	78.1 ± 14.9	+7.3 ± 2.0	0.003	83.5 ± 15.8	+2.9 ± 2.6	0.28	0.029
LDL-C (mg/dL)	81.7 ± 5.1	+3.0 ± 2.4	0.23	83.2 ± 5.5	-0.5 ± 2.5	0.83	0.66
ApoB100 (µg/mL)	64.4 ± 3.5	+3.0 ± 1.2	0.027	61.5 ± 3.7	+2.7 ± 0.7	0.002	0.09
ApoB100/ApoA1	1.42 ± 0.22	-0.02 ± 0.05	0.75	1.20 ± 0.18	+0.03 ± 0.05	0.62	0.003
ApoC3 (µg/mL)	243 ± 23	+5 ± 8	0.52	268 ± 22	+2 ± 10	0.82	0.09

[A]Baseline values are shown in "Day 1 8:00" and "Day 28 8:00". Values represent mean ± SEM

[B]"Δ Day 1" and "Δ Day 28" represent changes in 8:00 to 13:00 values during Day 1 and Day 28, respectively

[C]*P* values based of paired Student's *t*-test. The **Δ 8:00-8:00** *P* values lower than the Benjamini-Hochberg critical value are shaded gray

Supplemental Table 6. Blood Levels of Common Metabolites and Hormones

Metabolite (units)	Day 1	Day 28	Change
Glucose (mg/dL)	08:00: 87.6 ± 1.9 13:00: 83.1 ± 1.4 -4.4 + ± 2.1 P = 0.06	08:00: 89.2 ± 1.1 13:00: 84.5 ± 1.3 -4.7 ± 1.1 P = 0.001	P = 0.42 P = 0.27 P = 0.92
Insulin (mμU/mL)	08:00: 10.3 ± 0.9 13:00: 9.9 ± 0.8 -0.6 ± 0.6 P = 0.24	08:00: 11.8 ± 1.1 13:00: 8.2 ± 0.8 -3.1 ± 0.9 P = 0.006	P = 0.15 P = 0.34 **P=0.02**
NEFA (mEq/L)	08:00: 0.37 ± 0.02 13:00: 0.90 ± 0.05 +0.54 ± 0.04 P < 0.001	08:00: 0.36 ± 0.03 13:00: 0.65 ± 0.04 +0.30 ± 0.04 P < 0.001	P = 0.60 P < 0.001 **P < 0.001**
β-hydroxybutyrate (mM)	08:00: 0.029 ± 0.010 13:00: 0.291 ± 0.069 +0.274 ± 0.069 P = 0.002	08:00: 0.041 ± 0.007 13:00: 0.122 ± 0.029 +0.081 ± 0.030 P = 0.021	P = 0.44 P = 0.006 **P=0.002**
Lactate (mmol/L)	08:00: 0.93 ± 0.09 13:00: 1.12 ± 0.13 +0.19 ± 0.14 P = 0.20	08:00: 0.84 ± 0.06 13:00: 0.79 ± 0.04 -0.05 ± 0.07 P = 0.46	P = 0.29 P = 0.008 P = 0.08
Pyruvate (mg/dL)	08:00: 0.94 ± 0.05 13:00: 0.89 ± 0.04 -0.11 + ± 0.09 P = 0.46	08:00: 0.87 ± 0.06 13:00: 0.74 ± 0.05 -0.24 ± 0.10 P = 0.16	P = 0.28 P = 0.22 P=0.43
Total bile acids (μmol/L)	08:00: 2.99 ± 0.39 13:00: 0.91 ± 0.13 -2.13 ± 0.45 P = 0.001	08:00: 4.46 ± 0.80 13:00: 1.64 ± 0.32 -2.82 ± 0.64 P = 0.001	**P = 0.04** P = 0.04 P = 0.48
FGF19 (pg/mL)	08:00: 118.2 ± 22.5 13:00: 56.6 ± 6.6 -61.6 ± 18.7 P = 0.006	08:00: 119.2 ± 19.5 13:00: 73.9 ± 10.0 -45.3 ± 19.5 P = 0.04	P = 0.97 P = 0.15 P = 0.57
Norepinephrine (pg/mL)	08:00: 263 ± 26 13:00: 323 ± 34 +57 ± 23 P = 0.03	08:00: 231 ± 12 13:00: 336 ± 55 +107 ± 48 P = 0.05	P = 0.27 P = 0.96 P = 0.31
Epinephrine (pg/mL)	08:00: 19.5 ± 3.1 13:00: 21.8 ± 4.3 +3.9 ± 5.5 P = 0.58	08:00: 20.1 ± 3.9 13:00: 20.6 ± 4.4 -0.3 ± 3.0 P = 0.91	P = 0.73 P = 0.78 P = 0.54
Dopamine (pg/mL)	08:00: 13.5 ± 1.0 13:00: 12.5 ± 0.0 -1.0 ± 1.0 P = 0.34	08:00: 12.5 ± 0.0 13:00: 14.9 ± 1.6 +2.4 ± 1.6 P = 0.17	P = 0.34 P = 0.17 P = 0.09
T3, total (ng/dL)	08:00: 110.4 ± 7.3 13:00: 110.4 ± 7.0 -1.4 ± 1.2 P = 0.25	08:00: 115.4 ± 8.3 13:00: 112.7 ± 7.5 -2.8 ± 1.5 P = 0.09	P = 0.25 P = 0.94 P = 0.26
T4, free (ng/dL)	08:00: 1.14 ± 0.03 13:00: 1.22 ± 0.04 +0.06 ± 0.02 P = 0.01	08:00: 1.18 ± 0.04 13:00: 1.22 ± 0.04 +0.05 ± 0.02 P = 0.05	P = 0.17 P = 1.00 P = 0.62
TSH (μIU/mL)	08:00: 1.59 ± 0.24 13:00: 1.31 ± 0.23 -0.34 ± 0.11 P = 0.009	08:00: 1.98 ± 0.48 13:00: 1.40 ± 0.25 -0.58 ± 0.27 P = 0.05	P = 0.19 P = 0.57 P = 0.17

Guide			
Metabolite/Hormone	Difference on Day 1 08:00 and 13:00 *P value:*	Difference on Day 28 08:00 and 13:00 *P value:*	*P: Difference in 08:00 values* *P: Difference in 13:00 values* *P: Difference in Delta Day 1 vs. Delta Day 28*

P values calculated with a paired Student's t-test. Highlighted in light yellow are metabolites/hormones that had significant changes that may be related to WAT activation. Highlighted in brown are metabolites/hormones that had significant changes that may be related to BAT activation.

Supplemental Table 7. Blood Levels of Common Metabolites and Hormones

Metabolite (units)	Day 1	Day 28	Change
Total Protein (g/dL)	08:00: 6.61 ± 0.25 13:00: 7.08 ± 0.12 +0.27 ± 0.09 P = 0.01	08:00: 6.80 ± 0.14 13:00: 7.04 ± 0.12 +0.24 ± 0.07 P = 0.004	P = 0.44 P = 0.61 P = 0.77
Creatine Kinase (U/L)	08:00: 79.0 ± 18.4 13:00: 87.5 ± 19.3 -3.46 ± 4.50 P = 0.98	08:00: 78.8 ± 14.6 13:00: 71.8 ± 12.4 -3.15 ± 3.11 P = 0.33	P = 0.99 P = 0.97 P = 0.94
Growth Hormone (ng/mL)	08:00: 1.67 ± 0.53 13:00: 0.61 ± 0.19 -1.05 ± 0.72 P = 0.18	08:00: 1.16 ± 0.71 13:00: 0.62 ± 0.23 -0.54 ± 0.62 P = 0.40	P = 0.37 P = 0.74 P = 0.48
Glucagon (pmol/L)	08:00: 24.8 ± 2.1 13:00: 23.9 ± 2.7 -1.9 ± 3.7 P = 0.62	08:00: 29.1 ± 2.5 13:00: 22.1 ± 2.1 -8.1 ± 2.7 P = 0.012	P = 0.20 P = 0.81 P = 0.33
Cortisol (µg/dL)	08:00: 13.6 ± 1.3 13:00: 7.6 ± 0.9 -6.0 ± 1.0 P < 0.001	08:00: 13.1 ± 1.3 13:00: 7.0 ± 0.7 -6.1 ± 1.4 P = 0.001	P = 0.61 P = 0.29 P = 0.94
ACTH (pg/mL)	08:00: 22.9 ± 4.9 13:00: 14.9 ± 1.1 -8.0 ± 4.4 P = 0.09	08:00: 26.1 ± 4.2 13:00: 15.2 ± 1.7 -11.0 ± 3.9 P = 0.02	P = 0.55 P = 0.82 P = 0.61
PTH (pg/mL)	08:00: 36.5 ± 3.0 13:00: 39.3 ± 3.0 +2.7 ± 1.6 P = 0.11	08:00: 35.3 ± 2.9 13:00: 38.1 ± 3.4 +2.9 ± 2.1 P = 0.20	P = 0.55 P = 0.54 P = 0.95
aGhrelin (pg/mL)	08:00: 234 ± 45 13:00: 237 ± 47 +3.9 ± 28.2 P = 0.89	08:00: 316 ± 55 13:00: 414 ± 118 +98 ± 105 P = 0.37	P = 0.07 P = 0.11 P = 0.44
Leptin (ng/mL)	08:00: 15.1 ± 2.1 13:00: 12.1 ± 1.6 -3.0 ± 0.9 P = 0.004	08:00: 17.2 ± 2.3 13:00: 13.1 ± 2.0 -4.1 ± 0.6 P < 0.001	P = 0.07 P = 0.34 P = 0.21
Adiponectin (µg/mL)	08:00: 8.56 ± 1.16 13:00: 9.19 ± 1.27 +0.63 ± 0.23 P = 0.02	08:00: 11.56 ± 1.57 13:00: 11.81 ± 1.57 +0.25 ± 0.17 P = 0.18	**P = 0.001** P= 0.001 P = 0.07
FGF21 (pg/mL)	08:00: 701 ± 186 13:00: 842 ± 336 +141 ± 176 P = 0.44	08:00: 819 ± 234 13:00: 524 ± 161 -295 ± 98 P = 0.01	P = 0.15 P = 0.12 P = 0.06
PYY (pg/mL)	08:00: 54.6 ± 7.1 13:00: 27.4 ± 3.2 -27.1 ± 5.2 P < 0.001	08:00: 59.2 ± 8.9 13:00: 30.8 ± 3.3 -28.4 ± 6.8 P = 0.001	P = 0.58 P = 0.17 P = 0.86
aGLP-1 (pg/mL)	08:00: 1.35 ± 0.21 13:00: 0.51 ± 0.06 -0.84 ± 0.21 P = 0.001	08:00: 1.67 ± 0.29 13:00: 0.71 ± 0.10 -0.96 ± 0.25 P = 0.002	P = 0.22 P = 0.05 P = 0.64

Guide			
Metabolite/Hormone	Difference on Day 1 08:00 and 13:00 *P value:*	Difference on Day 28 08:00 and 13:00 *P value:*	*P: Difference in 08:00 values* *P: Difference in 13:00 values* *P: Difference in Delta Day 1 vs. Delta Day 28*

P values calculated with a paired Student's t-test. Highlighted in light yellow are metabolites/hormones that had significant changes that may be related to WAT activation. Highlighted in brown are metabolites/hormones that had significant changes that may be related to BAT activation.

Supplemental Table 8. Blood Levels of Common Metabolites and Hormones

Metabolite (units)	Day 1	Day 28	Change
aGIP (pg/mL)	08:00: 9.08 ± 1.68 13:00: 6.51 ± 0.89 -2.57 ± 1.75 P = 0.16	08:00: 13.91 ± 2.99 13:00: 8.14 ± 1.55 -5.76 ± 3.37 P = 0.11	P = 0.07 P = 0.30 P = 0.34
tGIP (pg/mL)	08:00: 34.2 ± 4.2 13:00: 25.2 ± 2.6 -9.0 ± 2.7 P = 0.005	08:00: 44.8 ± 5.5 13:00: 27.3 ± 3.1 -17.5 ± 5.7 P = 0.009	**P = 0.03** P = 0.42 P = 0.12
Total Cholesterol (mg/dL)	08:00: 162.8 ± 7.5 13:00: 166.1 ± 7.4 +3.3 ± 4.3 P = 0.46	08:00: 169.4 ± 8.0 13:00: 167.0 ± 8.3 -2.4 ± 4.2 P = 0.58	P = 0.11 P = 0.88 P = 0.27
Triglycerides (mg/dL)	08:00: 87.3 ± 7.3 13:00: 90.5 ± 8.8 +3.2 ± 3.9 P = 0.43	08:00: 86.0 ± 7.9 13:00: 81.9 ± 9.2 -4.4 ± 3.5 P = 0.23	P = 0.72 P = 0.08 **P = 0.01**
HDL-C (mg/dL)	08:00: 63.6 ± 5.6 13:00: 63.3 ± 5.0 -0.3 ± 1.7 P = 0.87	08:00: 69.0 ± 5.9 13:00: 67.9 ± 6.0 -1.0 ± 1.7 P = 0.56	**P = 0.001** P = 0.02 P = 0.70
ApoA1 (µg/mL)	08:00: 965 ± 93 13:00: 1012 ± 90 +48 ± 36 P = 0.21	08:00: 1075 ± 110 13:00: 1126 ± 117 +51 ± 29 P = 0.10	**P = 0.02** P = 0.03 P = 0.95
ApoE (µg/mL)	08:00: 78.1 ± 14.9 13:00: 85.4 ± 16.6 +7.3 ± 2.0 P = 0.003	08:00: 83.5 ± 15.8 13:00: 86.4 ± 15.7 +2.9 ± 2.6 P = 0.28	**P = 0.03** P = 0.76 P = 0.18
LDL-C (mg/dL)	08:00: 81.7 ± 5.1 13:00: 84.7 ± 5.8 3.0 ± 2.4 P = 0.23	08:00: 83.2 ± 5.5 13:00: 82.7 ± 5.5 -0.5 ± 2.5 P = 0.83	P = 0.66 P = 0.63 P = 0.27
ApoB100 (µg/mL)	08:00: 64.4 ± 3.5 13:00: 67.4 ± 3.5 +3.0 ± 1.2 P = 0.03	08:00: 61.5 ± 3.7 13:00: 64.2 ± 3.3 +2.7 ± 0.7 P = 0.002	P = 0.09 P = 0.05 P = 0.77
ApoB100/ApoA1 (ug/mL)	08:00: 1.42 ± 0.22 13:00: 1.40 ± 0.21 -0.02 ± 0.06 P = 0.75	08:00: 1.20 ± 0.18 13:00: 1.23 ± 0.20 +0.03 ± 0.05 P = 0.62	**P = 0.003** P = 0.008 P = 0.63
ApoC3 (µg/mL)	08:00: 243 ± 23 13:00: 248 ± 25 +6 ± 8 P = 0.52	08:00: 268 ± 22 13:00: 270 ± 22 +2 ± 10 P = 0.82	P = 0.09 P = 0.15 P = 0.77

Guide			
Metabolite/Hormone	Difference on Day 1 08:00 and 13:00 *P value:*	Difference on Day 28 08:00 and 13:00 *P value:*	*P: Difference in 08:00 values* *P: Difference in 13:00 values* *P: Difference in Delta Day 1 vs. Delta Day 28*

P values calculated with a paired Student's t-test. Highlighted in light yellow are metabolites/hormones that had significant changes that may be related to WAT activation. Highlighted in brown are metabolites/hormones that had significant changes that may be related to BAT activation.

Supplemental Table 9. Parameters of the Frequently-Sampled Intravenous Glucose Tolerance Test

Parameter	Unit	Day 0	Day 27	Change	*P* value[A]
Glucose AUC	mg dL^{-1} min	18952 ± 316	15518 ± 256	-3434	0.11
Insulin AUC	mIU L^{-1} min	6190 ± 497	5908 ± 744	-282	0.16
S_G	min^{-1}	1.84E-02 ± 0.2E-02	2.50E-02 ± 0.2E-02	+6.62E-03	0.002
S_I	mIU^{-1} L min^{-1}	3.33 ± 0.27	4.47 ± 0.46	+1.14	0.026
AIR_G	mIU L^{-1} min	655 ± 106	896 ± 158	+241	0.039
DI		1995 ± 302	3626 ± 596	+1631	0.005
Kg	min^{-1}	1.86 ± 0.18	2.70 ± 0.42	+0.84	0.036
GB	mg dL^{-1}	87.2 ± 1.8	88.2 ± 1.1	+1.0	0.60
IB	mIU L^{-1}	8.06 ± 0.82	8.45 ± 0.91	+0.39	0.68
P(2)	min^{-1}	6.96E-02 ± 0.4E-02	6.90E-02 ± 0.4E-02	-6.68E-04	0.87
P(3)	mIU L^{-1} min^{-2}	2.34E-05 ± 0.3E-05	3.15E-05 ± 0.4E-05	+8.1E-06	0.052
G(0)	mg dL^{-1}	257 ± 8	299 ± 13	+42	0.002
GEZI	min^{-1}	1.58E-02 ± 0.2E-03	2.14E-02 ± 0.2E-03	+5.63E-03	0.005
HOMA β-cell	mIU mM^{-1}	122.6 ± 10.6	122.9 ± 14.5	+0.3	0.98
HOMA IR	mM mU L^{-2}	1.75 ± 0.20	1.84 ± 0.20	+0.09	0.72

Abbreviations:
AUC = Area under the curve
S_G = Glucose effectiveness
S_I = Insulin secretion index
AIR_G = Acute insulin response to glucose
DI = Disposition Index
Kg = Glucose disappearance rate, 10-20 min after injection of glucose
GB = Basal glucose concentration
IB = Basal insulin concentration
P(2) = Rate constant describing a spontaneous decrease of tissue glucose uptake ability
P(3) = Insulin-dependent increase in tissue glucose uptake ability per unit of insulin concentration access over baseline
G(0) = Initial glucose concentration
GEZI = Glucose effectiveness at zero insulin
HOMA = Homeostatic model

[A]*P* value based on paired Student's *t*-test

Supplemental Table 10. BARCIST Criteria (from Table 1 of Chen KY et al. Cell Metab. 2016;24): 210–222.)

Participant Characteristics	Recommendation	Location in manuscript
Age, sex, ethnicity/race, height, weight, BMI	Report	Table 1
Lean (fat free) and fat body mass	Report (including method of determination)	Table 1
Prescription and over counter medications	Report	Described here: https://clinicaltrials.gov/ct2/show/NCT03049462
β-blockers, β-adrenergic agonists	Exclusion criterion	
Weight change of >5% within 3 months	Exclusion criterion (if weight change prior to the study is expected as part of the study design, consider using dynamic PET/CT FDG or the use of another tracer in combination with FDG)	
Habitual tobacco use	Exclusion criterion	
Habitual excessive alcohol use	Exclusion criterion	
Menstrual cycle phase, hormone replacement therapy use	Report Recommend that participants be studied at same phase if possible	
Pregnancy	Exclusion criterion	
Plasma Glucose	Exclude or control if >11mM	

Subject Preparation	Recommendation	Location in manuscript
Meals 24 hours before scan	Avoid high fat foods	Described in the Supplemental Methods section, headings “Metabolic Testing: Day 0 & Day 27” and “Quantification of Metabolic Activity, Physiological, and Clinical Measurements”
Caffeine 24 hours before scan	Not recommended	
Fast duration before scan	>6 hours	
Pharmaceuticals	Report	
Fasting plasma glucose (within 3 hours of tracer injection)	Report Should be <7mM. Do not proceed with experiment if >11mM	
Strenuous activity within 48 hours of scan	Not recommended	
Clothing during scan	Report thermal “R” insulation value (CLO) Examples of acceptable clothing: hospital gown, scrubs, tee shirt and shorts	
Environmental (room) temperature	Report if subject was exposed to cool temperatures within 12 hours of cooling period or scan	

Supplemental Table 11. BARCIST Criteria (from Table 1 of Chen KY et al. Cell Metab. 2016;24): 210–222.)

BAT Activation / Cooling Protocol	Recommendation	Location in manuscript
Fixed or personalized cooling paradigm	Report Recommend personalized paradigm if the study population is heterogeneous or if an intervention is used that is expected to change BAT volume or activation potential. Exposure conditions should be the same for repeated tests on a participant.	Not applicable as the BAT activation was done pharmacologically via 100 mg mirabegron
Cooling device	Report Recommend room air; water in cooling vest, suit, or blanket	
Air or Coolant temperature at shivering (if any)	Report Room air or water in cooling vest, suit, or blanket.	
Coolant temperature during cool period	Report both room air temperature and water temperature in cooling device, if used	
Total duration at cool temperature	Report Recommend minimum of 60 min. (after any incidence of shivering) prior to injection and ~60 min. after injection (until scan)	
Warm temperature (if applicable)	Report	
Duration at warm temperature (if applicable)	Report	
Method used to monitor skin temperature	Recommend surface temperature probes at multiple sites for continuous recordings.	
Method used to monitor shivering	Report Recommend EMG, observation, and/or self-report (in order of decreasing preference) Shivering should be minimized for 60 min. before and after tracer injection.	

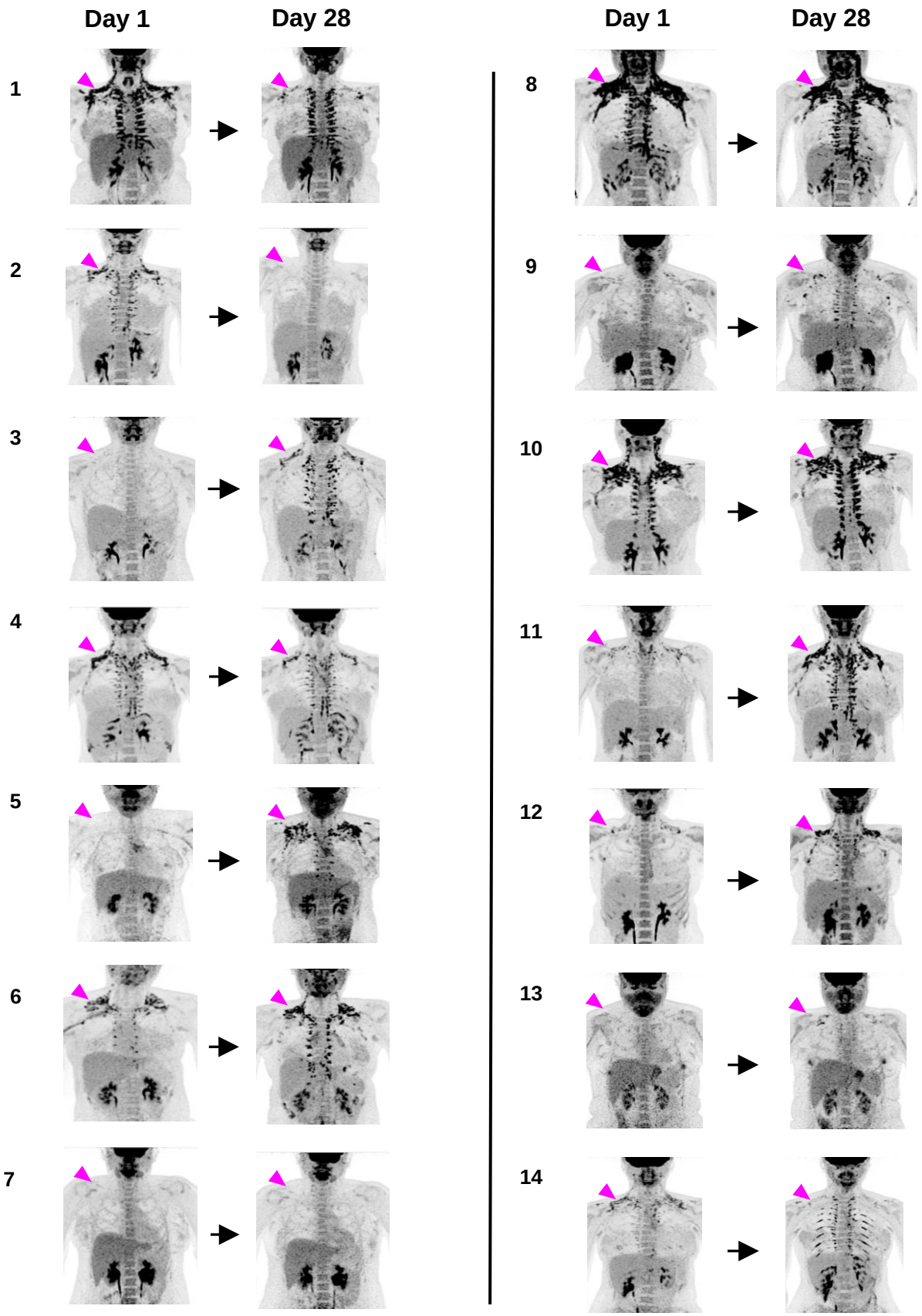
Supplemental Table 12. BARCIST Criteria (from Table 1 of Chen KY et al. Cell Metab. 2016;24): 210–222.)

PET/CT Examination	Recommendation	Location in manuscript
Manufacturer, model of PET/CT machine	Report Recommend using the same scanner for all scans within a study, especially for test/re-test in same participant	The methods used to quantify BAT metabolic activity and volume are described on lines 326-333. Additional details can be found in Leitner et al. {Leitner 2017}, and a step-by-step visual demonstration of the technique is described in Kim et al (58).
Data acquisition	Methods should be consistent with UPICT, QIBA and/or EANM standards.	
Reconstruction algorithms, reconstruction parameters and reconstruction software version used	Report Record software version number if possible, and recommend using the same software version for all images within a study	
FDG dose, site of injection	Report Recommend using a dose as low as possible for statistically valid imaging, with consideration for total dosage in repeat studies.	Peripheral iv, 185 MBq/5mCi
Method used to normalize FDG dose	Report Recommend using lean (fat free) body mass, Measured directly via densitometry, DEXA, or other validated method. If no direct measure is available, it can be estimated with Janmahasatian Formula	Lean body mass measured via DXA
Time between FDG injection and PET/CT scan (at cold temperature if cooling is used)	Report Recommend target 60 minutes with 55–70 minutes range	75-85 minutes
Time of day for scan	Report using 24-hour notation Recommend that all scans within a study be done at approximately the same time of day, if possible	14:15-14:25
Geographic location, time of year, outdoor temperature range	Report (latitude and longitude) For longitudinal interventional studies, recommend completing all scans within a single season or using an appropriate control group	Bethesda, MD, USA All times of year (Supplemental Fig.9)
Volume of water intake between injection and scan	Report Recommend drinking water be lukewarm such that participant perceives no difference between water and room temperature	Not recorded
Duration of PET scan	Report Recommend less than 60 min	20 minutes, 5 minutes for each of 4 bed positions
PET acquired voxel sizes and Field of View	Report Recommend that PET FOV to include the base of skull through inferior margin of liver, if possible	•PET/CT images were reconstructed into image voxels of 1.45 × 1.45 × 1.5 mm for PET and of 0.98 × 0.98 × 1.5 mm for CT •120 kVp, 115 mA
CT scan parameters, including kVp, acquired voxel sizes, tube power and Kv used, and Field of View	Report Recommend kVp = 120 ± 10 Recommend excluding pelvis to minimize radiation dose	
Extent and Duration of CT scan	Recommend base of skull to umbilicus, or as small as possible for study	<90 seconds

Supplemental Table 13. BARCIST Criteria (from Table 1 of Chen KY et al. Cell Metab. 2016;24): 210–222.)

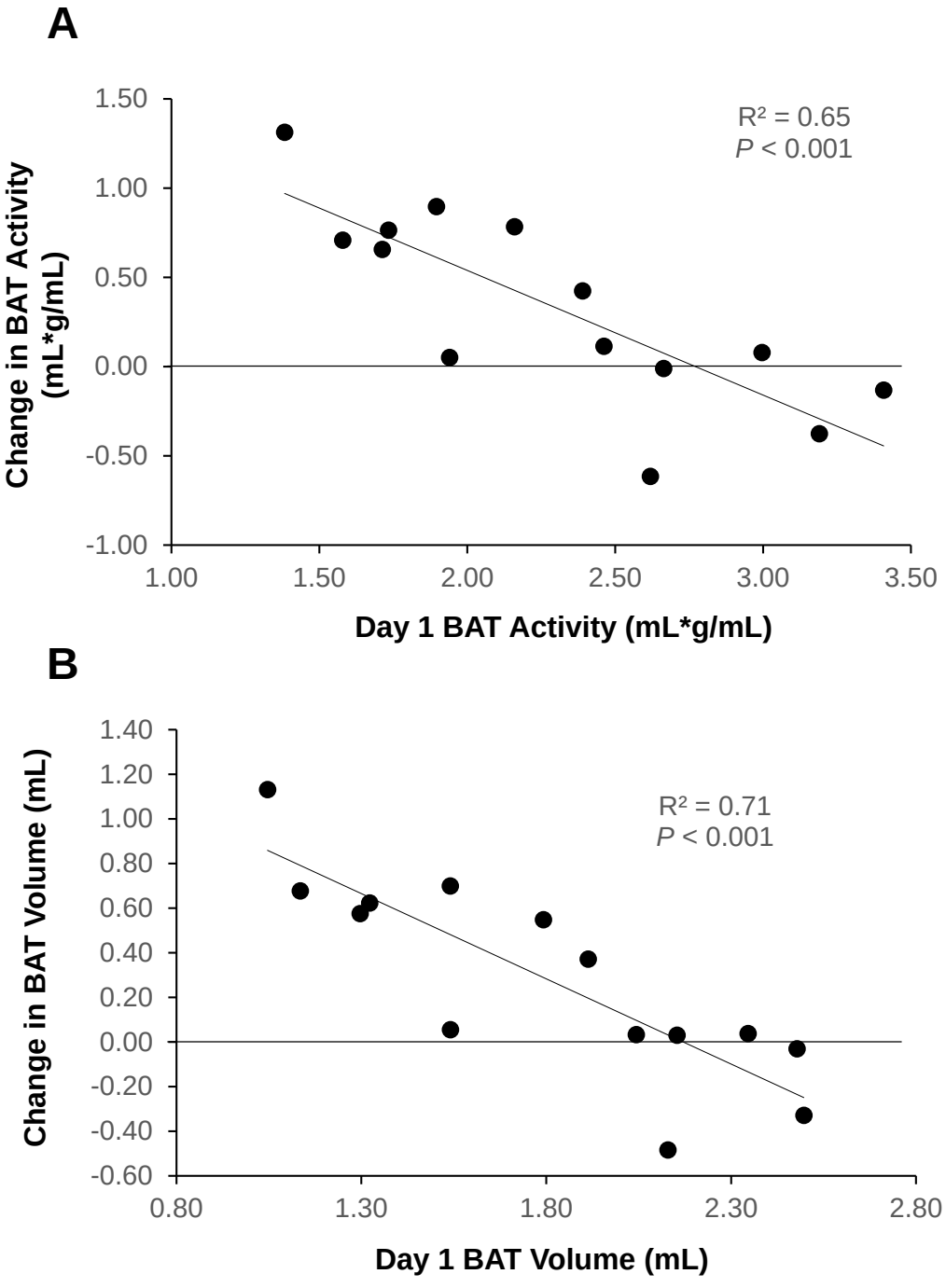
Data Analysis and Report	Recommendation	Location in manuscript
SUV and CT radiodensity scales	Continuous intensity scales should be used. SUV should be reported to 2 decimal places	Data are reported this way in Fig. 2 and Supplemental Figs. 2,3,5
SUV normalization	Recommend using lean (fat free) body mass to calculate SUV_{lean} (measured directly (i.e., DEXA). If no direct measure is available, can be estimated using Janmahasatian Formulation)	$SUV_{LBM} \geq$ (1.2g/mL) / (LBM%)
Minimum BAT metabolic activity threshold for calculation of BAT volume	$SUV_{lean} \geq 1.2$ If only the SUV_{bm} is available, convert to $SUV_{bm} \geq 1.2$ / (LBM/BM). (This approximates $SUV_{lean} \geq 1.2$ for lean young men of 20% body fat). Use measured LBM; if not available, use population-estimated LBM.	
Hounsfield Unit range used to define adipose tissue (WAT and BAT)	Report Recommend −190 to −10 for all fat (WAT + BAT). VOI should not include obvious non-fat tissues within this range such as lung	Range was -300 to -10 HU
BAT metabolic activity	Report $SUV_{bm/max}$ for the hottest single voxel in a VOI within BAT region, and $SUV_{bm/mean}$ for all voxels within BAT region. Report $SUV_{lean/max}$ for the hottest voxel within BAT region and $SUV_{lean/mean}$ for all voxels within BAT region. Report $SUV_{bm/peak}$ and $SUV_{lean/peak}$ for the hottest VOI within BAT region, for comparison between studies as this parameter is expected to vary less than SUV_{max}. Recommend reporting up to six VOI (hottest VOI in left and right supraclavicular region, left and right neck, left and right mediastinal)	BAT metabolic activity was determined and reported this way in Fig. 2 and Supplemental Figs. 1-5
BAT metabolic volume (BMV)	At a minimum, report BAT metabolic volume as the sum of all voxel volumes within suspected BAT region where $SUV_{bm} \geq 1.5$ and HU is between −190 and −10. It is recommended that a correction be made for body composition. Therefore, BMV should also be reported in one of two additional ways: • a) the sum of all voxel volumes within suspected BAT region where $SUV_{lean} \geq 1.2$ and HU is between −190 and −10 or • b) the sum of all voxel volumes within suspected BAT region where $SUV_{bm} \geq 1.2$ / (LBM/BM) and HU is between −190 and −10. Option b) is suggested for obese participants, although has not been validated. If a fixed volume or ‘mantle’ is used, describe the procedure for selecting it, as well as the volume size and location.	BAT metabolic volume was reported for all voxel volumes where $SUV_{LBM} \geq$ (1.2 g/mL)/(LBM%) where HU was between -300 and -10. It is shown if Fig. 2.
Reference tissue	Report reference tissue if used. To facilitate comparison among studies, recommend reporting mean normal tissue SUV in blood (descending aorta), 3 cm sphere in right lobe of liver (per PERCIST), and cerebellum if included in field of view.	No reference tissue was used.
Other data analysis as needed to assess experimental outcomes	Report	Not applicable

Supplemental Figure 1



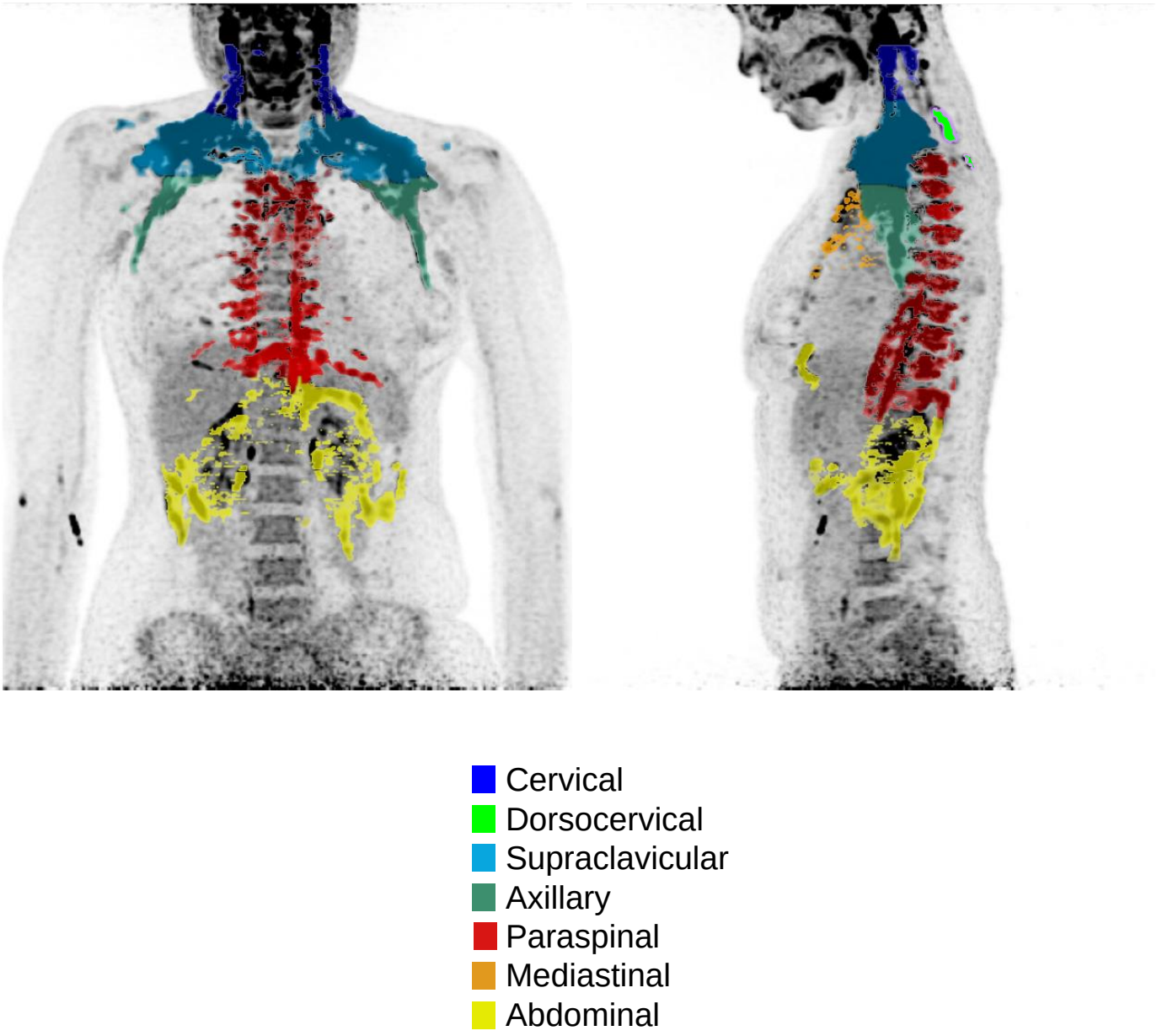
Supplemental Figure 1. Tissue glucose uptake in response to mirabegron after acute and chronic exposure. Shown are individual PET scans indicating ^{18}F-FDG uptake when given mirabegron on Day 1 (left) and on Day 28 (right). Magenta arrowheads point to the region of supraclavicular BAT.

Supplemental Figure 2



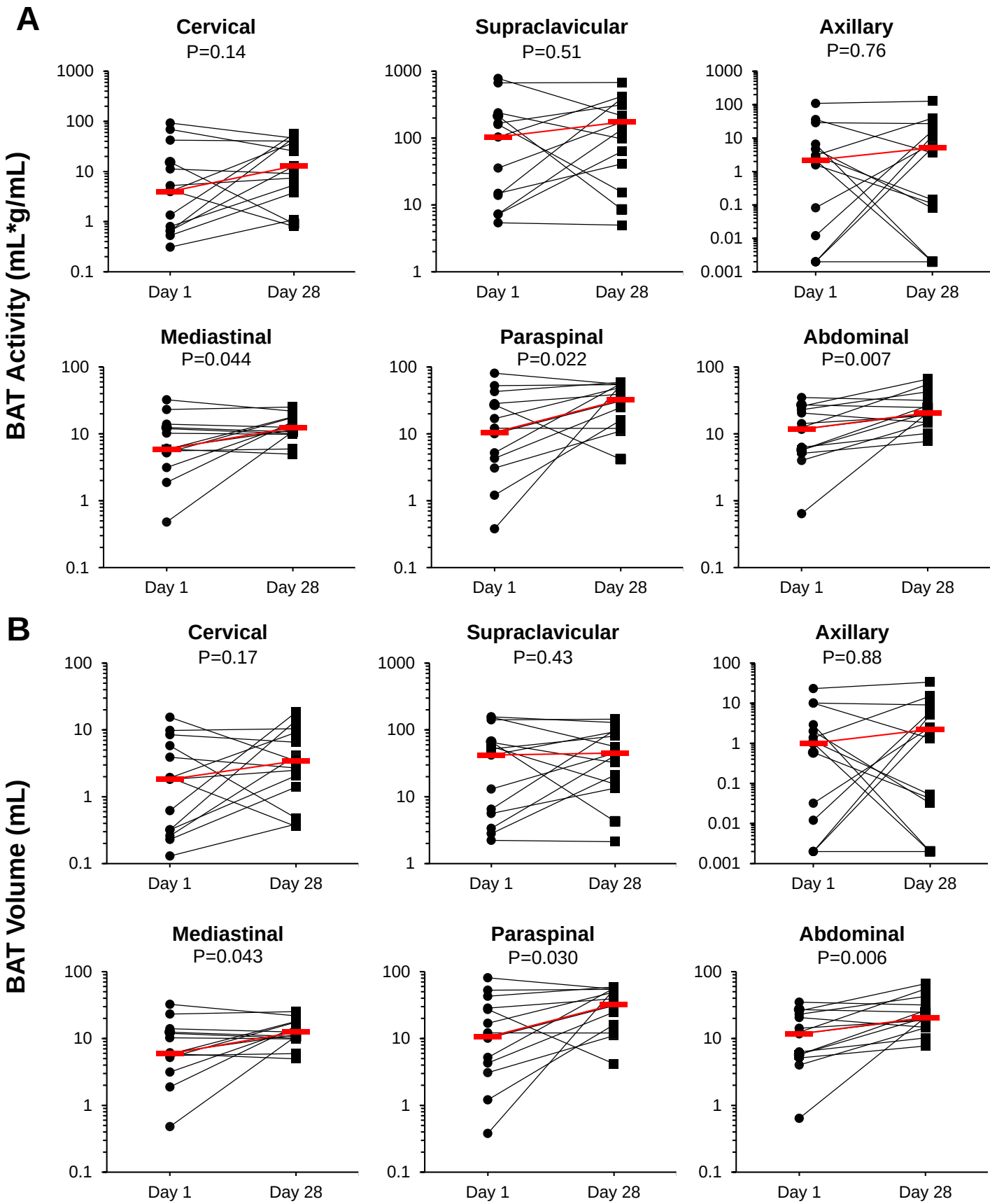
Supplemental Figure 2. Changes in BAT metabolic activity and volume as a function of Day 1 values. Change in detectable BAT (A) metabolic activity and (B) volume in subjects between Day 28 and Day 1 compared with initial values on Day 1. n=14.

Supplemental Figure 3



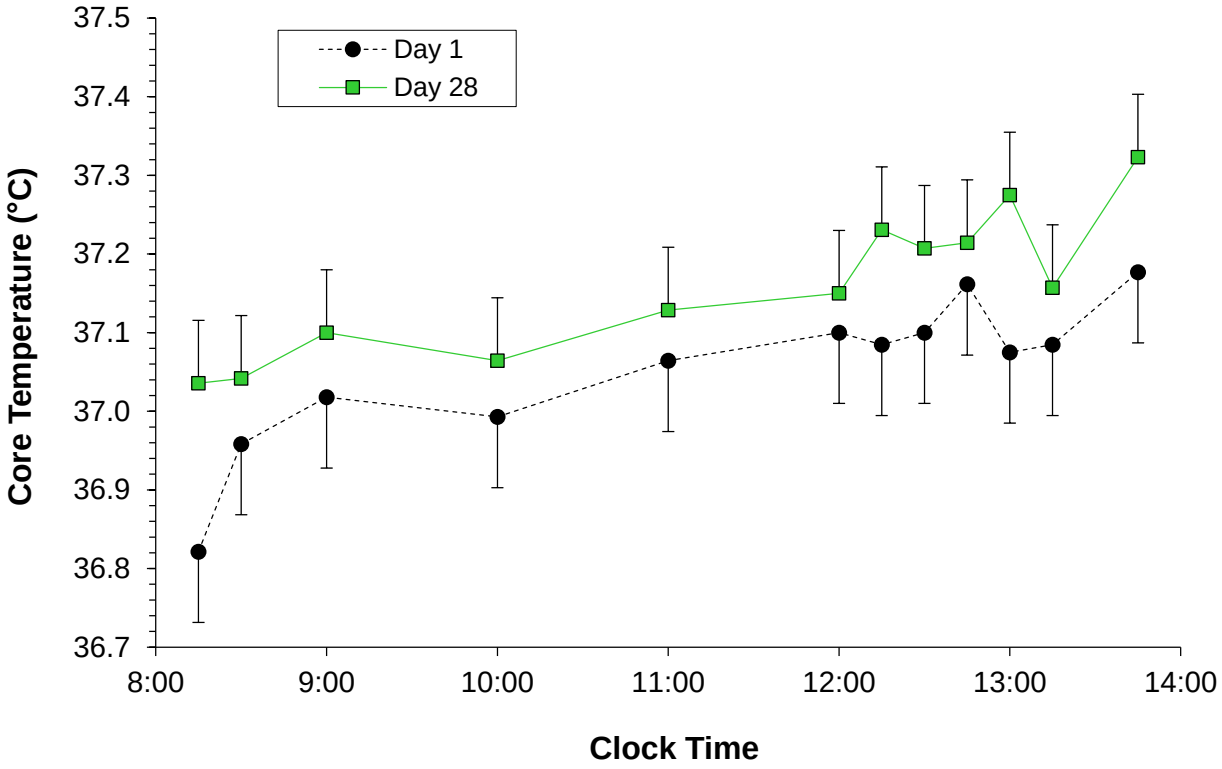
Supplemental Figure 3: BAT Depots. Depiction of seven BAT depots in a representative subject. Coronal view (left) sagittal view (right).

Supplemental Figure 4



Supplemental Figure 4: Changes in BAT metabolic activity and volume across depots. Individual depot changes in (A) BAT metabolic activity and (B) BAT volume in each of the six principal anatomical depots. Individual measures are Day 1 (black circles) and Day 28 (black squares). Red bars represent group medians. *P* values were determined via paired Student's *t*-test on log transformed data. n=14.

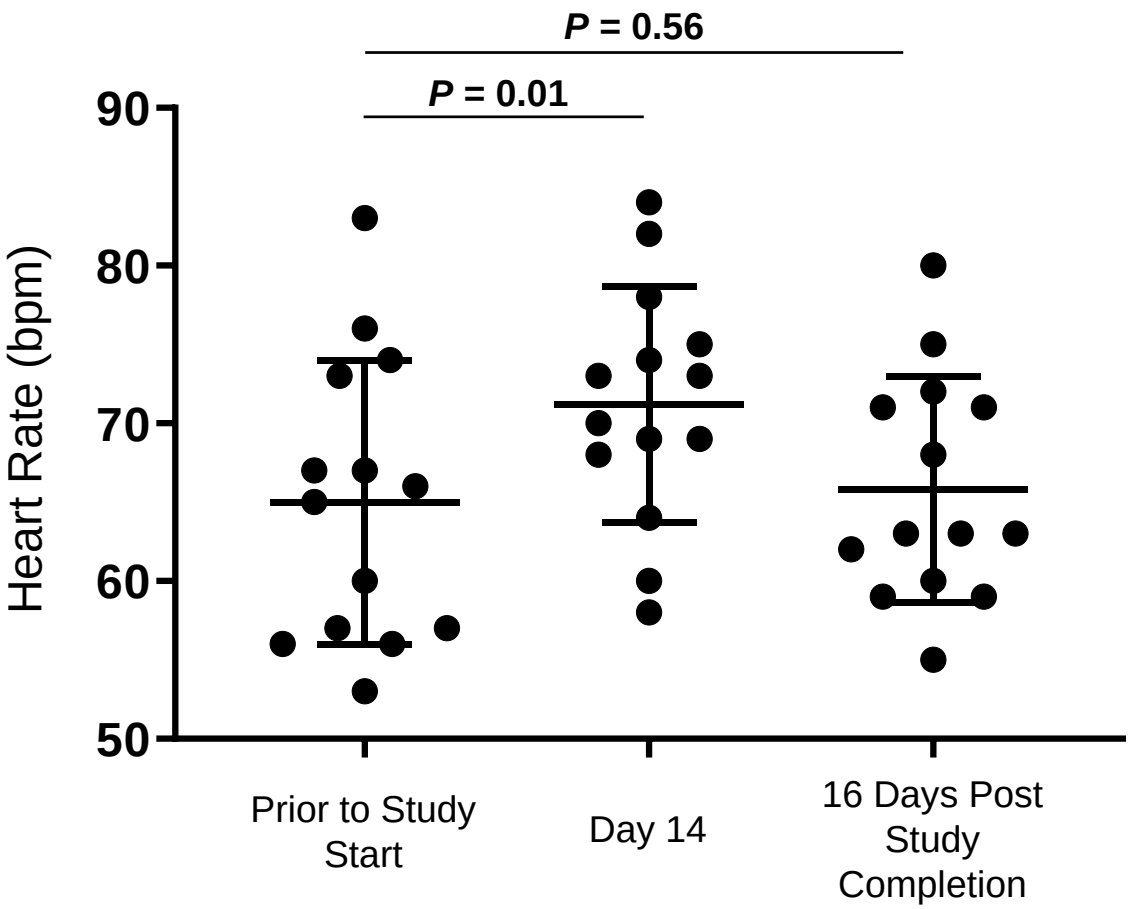
Supplemental Figure 5



Predictors of Core Temperature (Units)	*P* Value
Day of Study, 1 vs 28	<0.001
Time of Day	<0.001
Day of Study*Time of Day	0.79

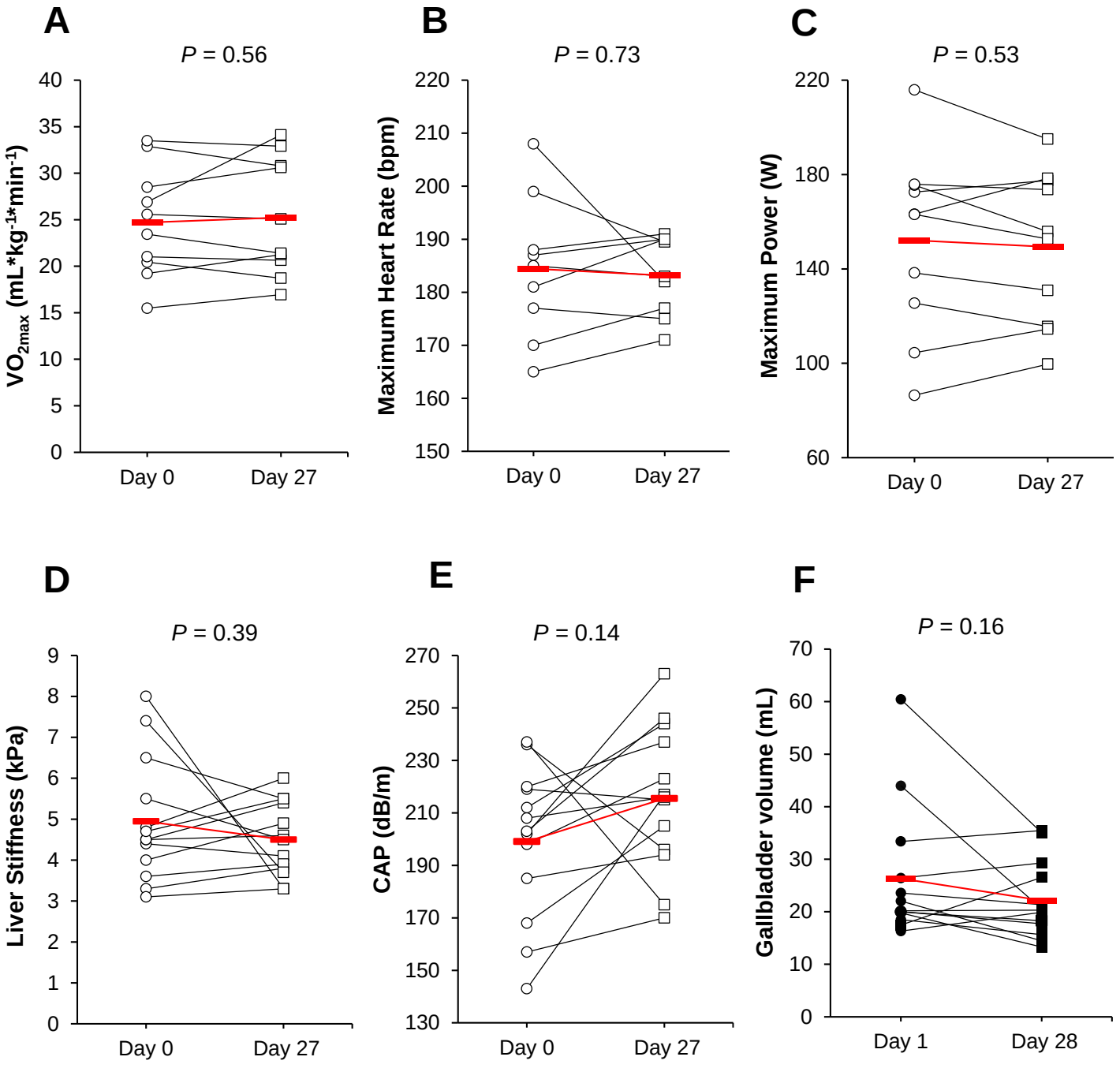
Supplemental Figure 5. Core Body Temperature. Subjects' mean **c**ore body temperature, measured via tympanic thermometer, while in the chamber on Day 1 (black circles) and Day 28 (green squares), error bars represent SEM. The effects of Day of Study, Time of Day, and their interaction were assessed using a linear mixed model, n=14.

Supplemental Figure 6



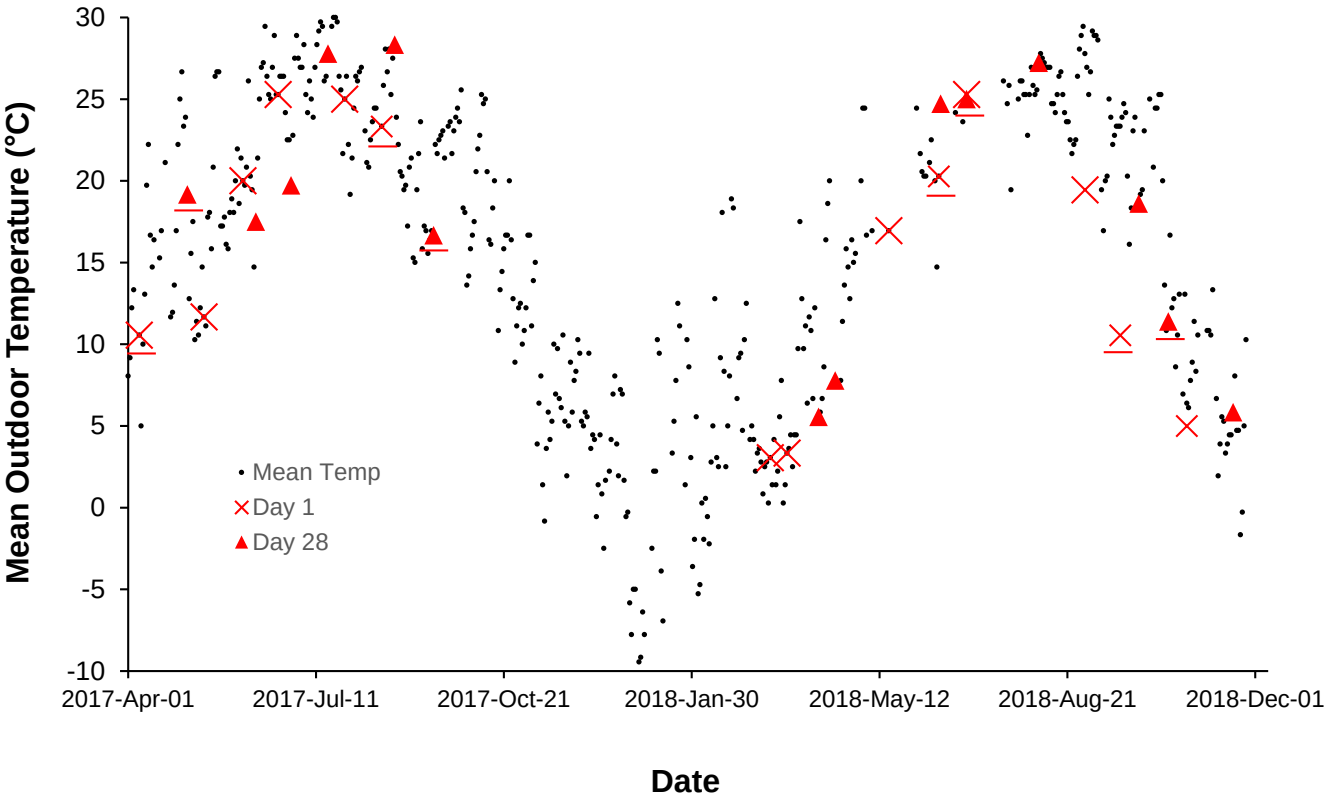
Supplemental Figure 6. Changes in heart rate. Heart rate, measured via electrocardiogram, at screening, interim (Day 14), and follow-up, which was 16 days after study completion and cessation of daily mirabegron dosing. *P* values were determined via paired Student's *t*-test. n=14.

Supplemental Figure 7



Supplemental Figure 7. Changes in exercise tolerance, liver parameters, and gallbladder size. Maximum (A) VO_{2max}, (B) heart rate, and (C) power achieved during maximal exercise tolerance test. Vibration controlled transient elastography assessment of (D) liver stiffness and (E) controlled attenuation parameter (CAP) for liver steatosis. (F) Individual gallbladder volume measured from CT scans. Symbols were used as follows: Day 0 measurements (white circles); Day 1 measurements (black circles); Day 27 measurements (white squares), Day 28 measurements (black squares). Red bars represent group means. *P* values were determined via paired Student's t-test. n=9-14.

Supplemental Figure 8



Supplemental Figure 8. Mean Outdoor Temperature in Bethesda, MD The mean outdoor temperatures over the course of the clinical trial are shown as black circles. The subjects' Day 1 study days are red "X" marks, and the Day 28 study days are red triangles. n=14. The symbols for the four subjects who traveled out of the area during their mirabegron treatment are underlined in red.

TREND Statement Checklist

Paper Section/ Topic	Item No	Descriptor	Reported? ✓	Reported? Pg #
Title and Abstract				
Title and Abstract	1	• Information on how unit were allocated to interventions	X	3
		• Structured abstract recommended	X	3
		• Information on target population or study sample	X	3
Introduction				
Background	2	• Scientific background and explanation of rationale	X	5
		• Theories used in designing behavioral interventions	X	5
Methods				
Participants	3	• Eligibility criteria for participants, including criteria at different levels in recruitment/sampling plan (e.g., cities, clinics, subjects)	X	16-17
		• Method of recruitment (e.g., referral, self-selection), including the sampling method if a systematic sampling plan was implemented	X	16-17
		• Recruitment setting	X	16
		• Settings and locations where the data were collected	X	17-23
Interventions	4	• Details of the interventions intended for each study condition and how and when they were actually administered, specifically including:		
		○ Content: what was given?	X	17-20
		○ Delivery method: how was the content given?	X	17-20
		○ Unit of delivery: how were the subjects grouped during delivery?	X	17-20
		○ Deliverer: who delivered the intervention?	X	17-20
		○ Setting: where was the intervention delivered?	X	17-20
		○ Exposure quantity and duration: how many sessions or episodes or events were intended to be delivered? How long were they intended to last?	X	17-20
		○ Time span: how long was it intended to take to deliver the intervention to each unit?	X	17-20
		○ Activities to increase compliance or adherence (e.g., incentives)	n/a	
Objectives	5	• Specific objectives and hypotheses	X	16
Outcomes	6	• Clearly defined primary and secondary outcome measures	X	16
		• Methods used to collect data and any methods used to enhance the quality of measurements	X	16-23
		• Information on validated instruments such as psychometric and biometric properties	X	17-23
Sample Size	7	• How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules	X	16
Assignment Method	8	• Unit of assignment (the unit being assigned to study condition, e.g., individual, group, community)	X	16-17
		• Method used to assign units to study conditions, including details of any restriction (e.g., blocking, stratification, minimization)	X	16-17
		• Inclusion of aspects employed to help minimize potential bias induced due to non-randomization (e.g., matching)	X	17

TREND Statement Checklist

Blinding (masking)	9	• Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to study condition assignment; if so, statement regarding how the blinding was accomplished and how it was assessed.	X	16
Unit of Analysis	10	• Description of the smallest unit that is being analyzed to assess intervention effects (e.g., individual, group, or community)	X	16-17
		• If the unit of analysis differs from the unit of assignment, the analytical method used to account for this (e.g., adjusting the standard error estimates by the design effect or using multilevel analysis)	n/a	
Statistical Methods	11	• Statistical methods used to compare study groups for primary methods outcome(s), including complex methods of correlated data	X	23
		• Statistical methods used for additional analyses, such as a subgroup analyses and adjusted analysis	X	23
		• Methods for imputing missing data, if used	n/a	
		• Statistical software or programs used	X	23
Results				
Participant flow	12	• Flow of participants through each stage of the study: enrollment, assignment, allocation, and intervention exposure, follow-up, analysis (a diagram is strongly recommended)	X	17-20
		○ Enrollment: the numbers of participants screened for eligibility, found to be eligible or not eligible, declined to be enrolled, and enrolled in the study	X	17
		○ Assignment: the numbers of participants assigned to a study condition	n/a	
		○ Allocation and intervention exposure: the number of participants assigned to each study condition and the number of participants who received each intervention	n/a	
		○ Follow-up: the number of participants who completed the follow-up or did not complete the follow-up (i.e., lost to follow-up), by study condition	X	17
		○ Analysis: the number of participants included in or excluded from the main analysis, by study condition	X	
		• Description of protocol deviations from study as planned, along with reasons	n/a	
Recruitment	13	• Dates defining the periods of recruitment and follow-up	X	17
Baseline Data	14	• Baseline demographic and clinical characteristics of participants in each study condition	X	6-11
		• Baseline characteristics for each study condition relevant to specific disease prevention research	n/a	
		• Baseline comparisons of those lost to follow-up and those retained, overall and by study condition	n/a	
		• Comparison between study population at baseline and target population of interest	n/a	
Baseline equivalence	15	• Data on study group equivalence at baseline and statistical methods used to control for baseline differences	n/a	

TREND Statement Checklist

Numbers analyzed	16	• Number of participants (denominator) included in each analysis for each study condition, particularly when the denominators change for different outcomes; statement of the results in absolute numbers when feasible	X	16-23 33-36
		• Indication of whether the analysis strategy was "intention to treat" or, if not, description of how non-compliers were treated in the analyses	n/a	
Outcomes and estimation	17	• For each primary and secondary outcome, a summary of results for each estimation study condition, and the estimated effect size and a confidence interval to indicate the precision	X	6-11
		• Inclusion of null and negative findings	X	6-11
		• Inclusion of results from testing pre-specified causal pathways through which the intervention was intended to operate, if any	n/a	
Ancillary analyses	18	• Summary of other analyses performed, including subgroup or restricted analyses, indicating which are pre-specified or exploratory	X	6-11
Adverse events	19	• Summary of all important adverse events or unintended effects in each study condition (including summary measures, effect size estimates, and confidence intervals)	X	9
DISCUSSION				
Interpretation	20	• Interpretation of the results, taking into account study hypotheses, sources of potential bias, imprecision of measures, multiplicative analyses, and other limitations or weaknesses of the study	X	12-15
		• Discussion of results taking into account the mechanism by which the intervention was intended to work (causal pathways) or alternative mechanisms or explanations	X	12-15
		• Discussion of the success of and barriers to implementing the intervention, fidelity of implementation	X	12-15
		• Discussion of research, programmatic, or policy implications	X	12-15
Generalizability	21	• Generalizability (external validity) of the trial findings, taking into account the study population, the characteristics of the intervention, length of follow-up, incentives, compliance rates, specific sites/settings involved in the study, and other contextual issues	X	12-15
Overall Evidence	22	• General interpretation of the results in the context of current evidence and current theory	X	12-15

From: Des Jarlais, D. C., Lyles, C., Crepaz, N., & the Trend Group (2004). Improving the reporting quality of nonrandomized evaluations of behavioral and public health interventions: The TREND statement. *American Journal of Public Health*, 94, 361-366. For more information, visit: http://www.cdc.gov/trendstatement/