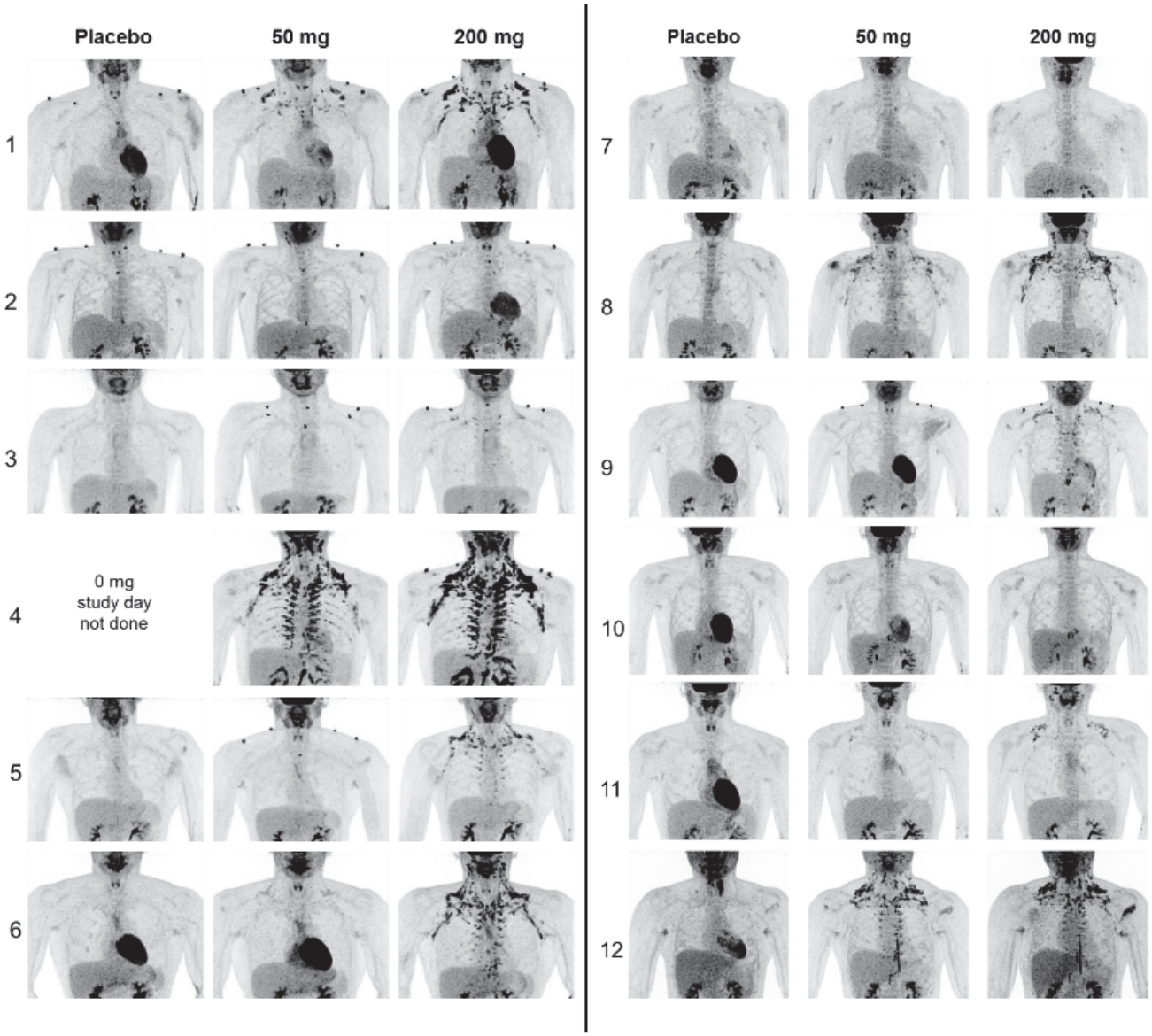
SUPPLEMENTARY DATA

Supplementary Figure 1. Each of the 12 subjects with detectable cold-activated BAT glucose uptake (left) were treated in random order with acute, one-time doses of placebo, 50 mg, and 200 mg mirabegron. Of note, subject #4 was unable to complete the study and did not receive the placebo dose.



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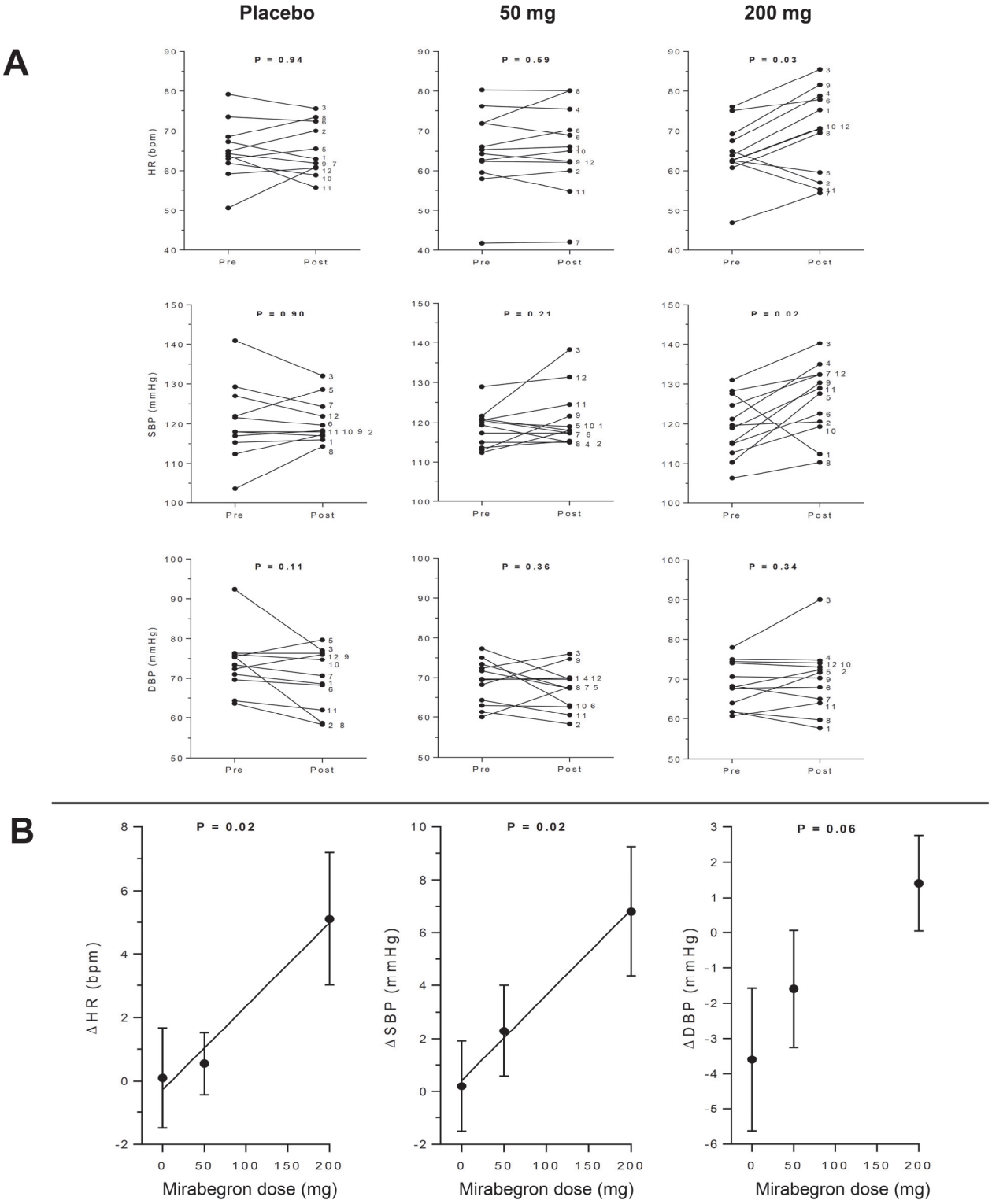
SUPPLEMENTARY DATA

Supplementary Figure 2. Vital Signs in Response to Mirabegron Treatment. (**A**) Changes in heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP) before and 5 hours after treatment with placebo, 50 mg, and 200 mg in 12 healthy male subjects. P values were determined by paired Student's *t*-test. (**B**) Dose-dependent changes in HR, SBP, and DBP. *P*values were determined by simple linear regression with the 12 subjects treated as random effects variables.



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Supplementary Table 1. PCR Information

Primer sequences used in quantitative RT-PCR for mouse tissue

Gene		Sequence
Actb	Forward	GGC ACC ACA CCT TCT ACA ATG
	Reverse	GGG GTG TTG AAG GTC TCA AAC
Adrb3	Forward	CCAGCCAGCCCTGTTGA
	Reverse	GGACGCGCACCTTCATAGC
Ucp1	Forward	CACCTTCCCGCTGGACACT
	Reverse	CCCTAGGACACCTTTATACCTAATGG

TaqMan Primer sequences used in quantitative RT-PCR for human tissue

Gene	Assay ID	Reference Sequence
ACTB	Hs99999903_m1	NM_001101.3
ADRB3	Hs00609046_m1	NM_000025.2
UCP1	Hs00222453_m1	NM_021833.4

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Supplementary Table 2. Mirabegron Pharmacokinetic Parameters

Pharmacokinetic Measure (Units)	50 mg	200 mg
C_{max}[a] ($ng*mL^{-1}$)	21.7 ± 13.4	142.6 ± 65.1
t_{max}[b] (min)	240 (180-360)	180 (120-300)
AUC_{0-300}[c] ($ng*h*mL^{-1}$)	215.6 ± 109.2	1,633.5 ± 591.2
$AUC_{240-300}$[d] ($ng*h*mL^{-1}$)	12.7 ± 6.2	70.2 ± 34.1

[a]Mean ± SD;
[b]Median (Range);
[c]AUC from time of administration to PET/CT scan reported as Mean ± SD;
[d]AUC during FDG uptake reported as Mean ± SD

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Supplementary Table 3. Predictors of BAT Metabolic Activity

Criterion	Predictor	P Value	β
BAT Activity	Dose	0.002	1.99
	C_{max}	0.010	2.02
	$AUC_{0\text{-}300}$	0.007	0.19
	$AUC_{240\text{-}300}$	0.015	3.92

Simple linear regression with random effects = Subject [Sequence], $n = 35$

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Supplementary Table 4. Mirabegron Cmax, AUC240-300, and BAT Activity

	C_{max} (ng/mL)			$AUC_{240-300}$ (ng*h/mL)			BAT Activity (mL*SUVmean*g/mL)		
Subject	Placebo	50 mg	200 mg	Placebo	50 mg	200 mg	Placebo	50 mg	200 mg
1	0.0	18.3	154.4	0.0	12.2	77.1	0.0	32.3	377.2
2	0.0	5.8	162.1	0.0	3.2	52.1	0.0	0.0	138.3
3	0.0	43.7	150.8	0.0	28.2	109.2	0.0	4.2	69.8
4	n/a	20.6	111.8	n/a	10.7	57.4	n/a	1529.5	3310.7
5	0.0	11.4	106.4	0.0	7.8	64.9	0.0	0.1	272.7
6	0.0	15.6	68.7	0.0	9.3	26.9	0.0	125.9	803.1
7	0.0	20.3	48.5	0.0	16.3	27.9	0.3	1.4	0.0
8	0.0	14.3	89.7	0.0	13.9	66.4	0.0	303.7	661.3
9	0.0	16.7	263.5	0.0	13.1	100.0	0.0	0.0	338.4
10	0.0	27.4	119.8	0.0	8.6	63.8	0.0	0.0	26.6
11	0.0	14.7	209.0	0.0	13.7	145.8	0.0	42.7	165.0
12	0.0	52.0	227.0	0.0	16.0	50.5	0.7	543.7	488.2
Mean ± SD	0.0 ± 0.0	21.7 ± 13.4	142.6 ± 65.1	0.0 ± 0.0	12.7 ± 6.2	70.2 ± 34.1	0.1 ± 0.2	215.3 ± 446.2	554.3 ± 903.4
Median (IQR)	0.0 (0.0-0.0)	17.5 (14.4-25.7)	135.3 (93.9-197.3)	0.0 (0.0-0.0)	12.7 (9.1 – 14.4)	64.4 (51.7 – 82.8)	0.0 (0.0-0.3)	18.3 (0.0-259.2)	305.6 (86.9-618.0)

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Supplementary Table 5. Blood Levels of Common Metabolites and Hormones

Metabolite (units)	Baseline[A]	Δ Placebo[B]	Δ 50 mg	Δ 200 mg
Glucose (mg/dL)	88 ± 7	-4 ± 6	-4[C] ± 6	-4 ± 10
Insulin (μU/mL)	6.4 ± 2.7	-2.6[C] ± 1.8	-0.8 ± 2.5	-1.1 ± 4.4
3-hydroxybutyrate (mM)	0.14 ± 0.07	0.04 ± 0.07	0.08[D] ± 0.07	0.08[C] ± 0.10
Lactate (mmol/L)	0.9 ± 0.3	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.5
Pyruvate (mg/dL)	0.8 ± 0.2	-0.1 ± 0.1	0.0 ± 0.2	0.0 ± 0.2
Glycerol (mg/mL)	7.74 ± 3.14	-1.37 ± 2.94	1.57[F] ± 3.75	0.14 ± 5.33
NEFA (mEq/L)	0.41 ± 0.15	0.06 ± 0.20	0.23[C] ± 0.29	0.28[D,F] ± 0.21
Total bile acids (μM)	4.56 ± 3.02	-3.32[D] ± 3.21	-2.02[D] ± 1.64	-3.29[E] ± 2.12
C4[G] (μM)	12.3 ± 11.8	-0.7 ± 10.3	-3.5 ± 14.3	0.5 ± 10.9
Norepinephrine (pg/mL)	339 ± 94	54 ± 94	17 ± 49	0 ± 120
Epinephrine (pg/mL)	22 ± 13	3 ± 15	-2 ± 13	4 ± 9
Dopamine (pg/mL)	36 ± 59	3 ± 11	-50 ± 174	-1 ± 4
T3, total (ng/dL)	116.0 ± 12.7	-0.7 ± 4.9	0.1 ± 9.1	-1.1 ± 10.5
T4, free (ng/dL)	1.3 ± 0.1	0.0[C] ± 0.1	0.1[C] ± 0.08	0.0 ± 0.1
TSH (μIU/mL)	2.31 ± 1.11	-0.62[D] ± 0.71	-0.50[D] ± 0.36	-0.77 ± 0.59
PTH (pg/mL)	33.2 ± 8.9	5.1 ± 9.0	3.2 ± 6.4	2.9 ± 6.9
Cortisol (μg/dL)	14.3 ± 3.0	-6.3[C] ± 3.9	-6.6[D] ± 4.7	-6.6[E] ± 4.6
Growth Hormone (ng/mL)	0.44 ± 0.88	2.71[C] ± 3.4	-0.23 ± 2.86	0.32 ± 1.13
Glucagon (pmol/L)	6.67 ± 1.95	-2.10 ± 3.33	-0.75 ± 2.51	-1.67 ± 3.02
ACTH (pg/mL)	32.1 ± 17.2	-15.2[C] ± 19.9	-11.9[B] ± 12.3	-16.4[C] ± 23.3
Leptin (pg/mL)	1102 ± 710	-311[D] ± 223	-444[D] ± 374	-367[D] ± 309
Adiponectin (ng/mL)	4473 ± 1672	92 ± 279	-178 ± 374	95 ± 288
Ghrelin (pg/mL)	37.9 ± 37.6	5.2 ± 27.6	20.3 ± 42.5	0.6 ± 40.4
GIP (pg/mL)	58.8 ± 28.7	-13.6 ± 45.2	-23.0[D] ± 19.9	-29.0[D] ± 23.5
PYY (pg/mL)	74.3 ± 29.5	-3.7 ± 28.23	-11.4[D] ± 12.4	-9.9 ± 17.2
GLP-1 (pg/mL)	2.06 ± 1.02	-1.71[C] ± 1.85	-0.91[D] ± 0.80	-1.35[E] ± 0.87
FGF19 (pg/mL)	197 ± 112	-118[D] ± 110	-88[C] ± 112	-102[E] ± 81
FGF21 (ng/mL)	99.8 ± 67.0	-52.5[C] ± 65.0	-41.4[D] ± 41.8	-27.8[D] ± 28.4
Total Protein (g/dL)	7.1 ± 0.2	0.2 ± 0.4	0.2[C] ± 0.3	0.2 ± 0.4
Creatine Kinase (U/L)	425 ± 606	-136 ± 339	-15 ± 22	-26 ± 47

[A]Baselinevalues reflect an average of placebo and active drug day pre-treatment values (RM-ANOVA confirmed pre-treatment values were not statistically different across days);
[B]BaselineValues are means ±SD
[C]Difference(Δ = post-treatment –pre-treatment) values ($P < 0.05$), Paired t-test.
[D]Difference(Δ = post-treatment –pre-treatment) values($P < 0.01$), Paired t-test.
[E]Difference(Δ = post-treatment –pre-treatment) values($P < 0.001$), Paired t-test.
[F]Differencebetween changes during placebo day and changes during active drug day ($P < 0.05$). Paired t-test. Depicted in gray. RM-ANOVA showed a dose-dependent relationship ($P = 0.05$).
[G]7α-hydroxy-4-cholesten-3-one

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Supplementary Table 6. Predictors of Cardiovascular Changes

	Placebo	50 mg	200 mg
ΔHeart Rate[A] (bpm)	+0.1 ± 5.2 P = 0.94	+0.5 ± 3.4 P = 0.59	+5.1 ± 7.2 P = 0.03
ΔSystolic BP[A] (mmHg)	+0.2 ± 5.7 P = 0.90	+2.3 ± 5.9 P = 0.21	+6.8 ± 8.4 P = 0.02
ΔDiastolic BP[A] (mmHg)	-3.6 ± 6.7 P = 0.11	-1.6 ± 5.8 P = 0.36	+1.4 ± 4.7 P = 0.34
ΔRPP[A] (mmHg*bpm)	+29.3 ± 236.2 P = 0.90	+223.3 ± 218.4 P = 0.33	+1,095.1 ± 304.0 P = 0.004

[A]Post (+250 min) – pre (-35 min) drug dosing, which are also the times of cold-day measurements;
[B]Difference of 20-min averages (post-treatment – pre-treatment). Paired t-tests.

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Supplementary Table 7. Predictors of Energy Expenditure

Criterion	Predictor	β	P Value
Change in REE[A] (kcal/h)	Dose	0.03	0.012
	C_{max}	0.02	0.068
	$AUC_{0\text{-}300}$	0.002	0.046

[A]Simple linear regression, with subject within sequence as the random effects variable, *n* = 34

ΔREE Predictors[A]	Simple Linear Regression			Multiple Linear Regression		
	β	R^2	P Value	β	$R^2_{adjusted}$	P Value
ΔRPP	0.0031	0.45	< 0.001	0.0024	0.44	0.003
BAT Activity	0.0038	0.17	0.002	0.0030		0.014
ΔHR	0.45	0.12	0.008	--	--	--
ΔSBP	0.33	0.35	0.009	--	--	--
ΔDBP	0.14	NS	0.399	--	--	--

[A]Regressions done with subject within sequence as the random effects variable, *n* = 34

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Supplementary Table 8. Means Comparisons for Mirabegron Dose and Bile Acids

		Cholic Acid		Deoxycholic Acid		Glycocheno-deoxycholate		Glycocholic Acid		Glycodeoxy-cholate		Taurodeoxy-cholic acid	
Bi-variate fit with dose		*P* = 0.43		*P* = 0.23		*P* = 0.009		*P* = 0.006		*P* = 0.03		*P* = 0.01	
Condition	Condition	Difference	P Value	Difference	P Value	Difference	P Value	Difference	P Value	Difference	P Value	Difference	P Value
Baseline	Placebo	0.898 ± 0.293	0.0158	0.911 ± 0.298	0.0166	0.805 ± 0.226	0.0037	0.695 ± 0.249	0.0341	0.735 ± 0.235	0.0132	0.736± 0.260	0.0307
Baseline	50mg	1.170 ± 0.283	0.0006	1.193 ± 0.289	0.0006	1.548 ± 0.219	<.0001	1.462 ± 0.241	<.0001	1.533 ± 0.227	<.0001	1.358 ± 0.252	<.0001
Baseline	200mg	1.179 ± 0.275	0.0003	1.012 ± 0.280	0.0032	1.787 ± 0.212	<.0001	1.621 ± 0.234	<.0001	1.720 ± 0.220	<.0001	1.565 ± 0.245	<.0001
Placebo	50mg	0.272 ± 0.354	0.869	0.282 ± 0.361	0.863	**0.743 ± 0.274**	**0.0408**	0.768 ± 0.302	0.063	**0.797 ± 0.284**	**0.0323***	0.623 ± 0.316	0.208
Placebo	200mg	0.280 ± 0.348	0.851	0.101 ± 0.355	0.992	**0.982 ± 0.269**	**0.0028**	**0.926 ± 0.296**	**0.0136**	**0.985 ± 0.279**	**0.0041**	**0.829 ± 0.310**	**0.0448**
50mg	200mg	0.009 ± 0.340	1.000	0.181 ± 0.347	0.954	0.239 ± 0.263	0.800	0.159 ± 0.290	0.947	0.187 ± 0.273	0.902	0.207 ± 0.303	0.903

Shown are means comparisons reporting all pairs using Tukey-Kramer honest significant difference (HSD). Comparisons with placebo where $P < 0.05$ are shown in bold.

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