RM-493, a Melanocortin-4 Receptor (MC4R) Agonist Increases Resting Energy Expenditure in Obese Individuals

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Context: Activation of MC4R with the synthetic agonist RM-493 decreases body weight and increases energy expenditure (EE) in non-human primates. The effects of MC4R agonists on EE in humans have not been examined to date.

Objective:, Design, and Setting: In a randomized, double-blind, placebo-controlled, crossover study, we examined the effects of the MC4R agonist, RM-493, on resting EE (REE) in obese subjects in an inpatient setting.

Study Participants and Methods: Twelve healthy adults (6 M, 6 F) with BMI 35.7 $\pm$ 2.9 kg/m^2 (mean, SD) received RM-493 (1 mg/24 h) or placebo by continuous subcutaneous infusion over 72 hours, followed immediately by crossover to the alternate treatment. All subjects received a weight-maintenance diet (50% carbohydrate, 30% fat, 20% protein) and performed 30 minutes of standardized exercise daily. Continuous EE was measured on the third treatment day in a room calorimeter and REE in the fasting state was defined as the mean of two 30-minute resting periods.

Results: RM-493 increased REE vs. placebo by 6.4% (95% CI: 0.68 to 13.02 %), on average by 111 kcal/24h (95% CI: 15 to 207 kcal, p=0.03). Total daily EE trended higher while the thermic effect of a test meal and exercise EE did not differ significantly. The 23-h non-exercise respiratory quotient was lower during RM-493 treatment (0.833 $\pm$ 0.021 vs. 0.848 $\pm$ 0.022, p=0.02). No adverse effect on heart rate or blood pressure was observed.

Conclusions: Short-term administration of the MC4R agonist, RM-493 increases REE and shifts substrate oxidation to fat in obese individuals.

The central melanocortin system, comprised of melanocortins (MCs), agouti, agouti-related proteins and their receptors (MCRs), integrates neural, metabolic, and hormonal signals serving a critical role in the maintenance of body weight (1, 2). The MCs are a family of peptide hormones, including α-melanocyte-stimulating hormone (MSH), β-MSH, γ-MSH, and adrenocorticotropic hormone (ACTH) derived from a common precursor, proopiomelanocortin (POMC). Activation of MC subtype 4 receptors (MC4Rs) in the hypothalamus in animal models reduces food intake, increases energy expenditure, and causes weight loss when given chronically (3). In humans, the most frequent monogenic etiology of obesity is haploinsufficiency of the *MC4R*, highlighting a role for the MC4R in energy homeostasis (4). This is exemplified in the Pima Indian population with a high prevalence of

Abbreviations:

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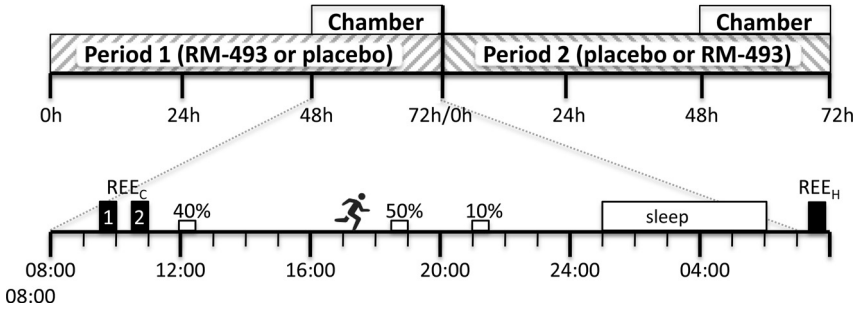

Figure 1. Study design. Subjects received 72-hour infusions of RM-493 or placebo and then immediately were crossed over to the other treatment. In each period, after 48-hour of treatment, subjects entered the metabolic chamber at 8am. REE_C was measured at 09:30–10:00 and 10:30–11:00, meals were at 12:00 (40% of kcal), 18:30 (50% of kcal), and 21:00 (10% of kcal), exercise was at 17:00–17:30, and REE_H was measured after leaving the chamber.

MC4R loss of function variants, associated with obesity, type 2 diabetes mellitus and lower 24-hour, resting and sleeping energy expenditure (5). Polymorphisms near MC4R also contribute to common obesity (6). Thus, MC4R is an attractive target for the treatment of obesity (7).

The five MCR subtypes have diverse expression and binding profiles, both in the central nervous system (CNS) and peripherally, and play a role in the regulation of sexual function, pigmentation, inflammation, analgesia, immunomodulation, blood pressure (BP), and steroidogenesis in addition to energy homeostasis (8). Several synthetic MC4R agonists have reached clinical trial, but each suffered from either a lack of efficacy or from cardiovascular adverse effects (7). The small peptide MC4R agonist RM-493, administered by subcutaneous infusion for eight weeks in obese rhesus macaques, decreased food intake, reduced body weight, and improved glucose tolerance (9). The initial decrease in food intake coupled with a sustained increase in energy expenditure (EE) caused 13.5% weight loss over 8 weeks, without adverse cardiovascular effects. Whether agonists of the MC4R pathway similarly increase EE in humans is not known. Here we test if brief administration of RM-493 increases resting energy expenditure (REE) in obese human subjects.

Subjects and Methods

Study Design and Study Subjects

The Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) approved the study protocol (ClinicalTrials.gov identifier NCT01867437). Written informed consent was obtained from all subjects. Twelve volunteers (6 male, 6 female) in general good health between the age of 18 and 50 years with a body mass index (BMI) between 30 and 40 kg/m^2 were enrolled. Subjects with diabetes, hypertension, liver enzymes more than 1.5 times the upper limit of normal, thyroid dysfunction, symptomatic sleep apnea, or recent illness, pregnancy, cancer or surgery were excluded at the initial screening visit.

Eligible subjects were admitted to the Metabolic Clinical Research Unit at the NIH Clinical Center for an 8-day inpatient stay (Figure 1). On the day of admission, subjects started a weight-maintenance diet (50% carbohydrate, 30% fat, and 20% protein); consumption of caffeine, alcohol, and tobacco use was prohibited. Each subject exercised daily for 30-minutes on a treadmill at the same self-selected settings (speed and grade) and was continuously monitored with triaxial accelerometers (Actigraph GT3X+, Actigraph LLC, Pensacola, FL) on the wrist and hip, measuring spontaneous and volitional physical activity. Body weight, BP and pulse were measured daily in the fasting state. Subjects were randomized to receive RM-493 1.0 mg/24-hour or placebo for 72-hour by continuous, subcutaneous infusion using an insulin pump (OmniPod® Insulet, Bedford, MA) starting at 00:80 on the second day of admission (period 1). At the end of this infusion, the subjects were directly crossed over to a 72-hour infusion with the other treatment (period 2). After completion of the final assessments of period 2, subjects completed the study and were discharged.

Study procedures

Energy expenditure measurements and substrate oxidation

Each subject underwent continuous recordings of EE and respiratory quotient (RQ) in a whole-room indirect calorimeter (referred to subsequently as a metabolic chamber) at thermoneutrality (24.6 ± 0.7°C) on the third day of each treatment period. The subjects were fasting from 20:00 and entered the chamber at 08:00 for 23-hour, as described (10–12). The primary outcome, REE_C was defined as the average resting EE in the metabolic chamber from 09:30 (49.5-hour after starting treatment) to 10:00 and 10:30 to 11:00 while the subject was fasting, completely still, and awake in a seated position under the observation of a member of the research team. REE defined in our study and basal metabolic rate (BMR) are very similar in important aspects (performed after 10–12 hour fast, at thermoneutrality, awake, at rest without physical and psychological stress) but differ slightly in posture (semirecumbent sitting vs supine). REE is a precise surrogate for BMR (10). By allowing the subject to acclimate to the chamber for 90 minutes and then using two 30-minute measurement periods, we achieve a coefficient of variation of 2.5%, which was similar to previous metabolic chamber REE measurements (11), maximizing sensitivity to detect changes in REE_C. The 30-minute interval between sessions allows subject movement, improving compliance during the measurement periods. The semirecumbent position of the subject allowed direct observation of the subject to assure that they did not fall asleep.

Subjects consumed a standardized test meal containing 40% of their daily energy requirements from 11:30 to 12:00. The thermic effect of food (TEF) was assessed by averaging data

collected from 11:30–15:00. Exercise EE was assessed during the subject's daily 30-minute of treadmill walking. Sleeping EE was assessed from 00:00 to 04:00. Data collected during the periods of REE_C and SEE were excluded if microwave activity was > 2% (24% of the SEE and 3% of the REE data were excluded) (12). Total energy expenditure (TEE) and average RQ of the 23-hour chamber period excluded the 30-minute exercise period.

As a secondary measure, REE_H was measured using a hood indirect calorimetry cart system (Parvo Medics TrueOne® 2400, Sandy, UT) immediately after exiting the chamber in fasted and resting state.

Body composition. Total body fat content, total fat mass, and fat free mass were measured using dual-energy X-ray absorptiometry scanner (Lunar iDXA, GE Healthcare, Madison, WI, USA).

MC4R genotyping. The coding regions of *MC4R* were sequenced at Eurofins Genomics (Louisville, KY).

Biochemical measurements. Blood was drawn daily after an overnight fast. All clinical chemistry testing was performed by the Department of Laboratory Medicine, Clinical Center, NIH. Glucose, insulin, c-peptide, total glucagon-like peptide-1 (GLP-1), free fatty acids (FFA), triglycerides, TSH, total T4, prolactin, and 24-hour urine cortisol, irisin, total ghrelin, FGF-21, and total peptide YY (PYY) were measured as described in supplemental material. Total T3, free T3, and free T4 were measured by LC-MS/MS with electrospray ionization (Agilent 6460 mass spectrophotometer and Agilent 1200 HPLC, Agilent Technologies, Wilmington, DE).

Statistical analysis

Descriptive analysis results are reported as mean and standard deviation, if not specified. Comparisons of responses between pre- and post-treatment were performed using paired *t* test for normal continuous variables. Normality was checked using Q-Q plots before comparisons and skewed variables were logarithm transformed. Based on coefficient of variation of 2.5%, the study had, a priori, 80% power to detect a difference of 2.5% in REE_C with 12 subjects. In comparison, REE_H (5.4% CV for ParvoMedic cart) requires 39 subjects to detect this difference (13).

Mixed model regression was used to identify the association between the repeated measurements of response variables with treatment, with adjusting for baseline, for glucose, insulin, c-peptide, FFA, triglycerides, total GLP-1, hormones, REE_H, and cardiovascular parameters (SBP, DBP, HR). For metabolic variables we included data from fasting daily blood draws corresponding to 24, 48, and 72-hour of drug or placebo. The REE_H and vital signs obtained at 72-hour, the maximal duration of treatment, were analyzed. Absence of treatment-by-period interaction effects were confirmed for all variables studied.

Analyses were performed using SAS (version 9.3; SAS Institute, Cary, NC) and JMP (version 10.0; SAS Institute, Cary, NC) with significance being $P < .05$. All statistical testing was two-sided and not corrected for multiple comparisons.

Results

Baseline clinical characteristics of study subjects. This study used a cross-over design where each study participant randomly received either placebo or RM-493 and then was crossed to the alternate treatment. Fifteen subjects were screened and all of the 12 eligible subjects were randomized and completed the study (Supplemental figure 1). Baseline clinical characteristics are reported in Table 1. All participants were weight-stable, normotensive, nondiabetic, and had normal thyroid function at baseline. Participants were obese (Grade I and II; 35.7 ± 2.9 kg/m^2; 104 ± 10 kg), with a body fat of 49.2 ± 1.5% in the women and 32.5 ± 6.8% in the men. No subjects had exon nucleotide variants of *MC4R* associated with obesity (Supplemental material).

Administration of placebo and RM-493. Placebo or RM-493 was administered as a continuous subcutaneous infusion during each 72-hour treatment period. Plasma RM-493 levels at 24-hour (5.88 ± 0.59 ng/mL) were similar to those at 72-hour of treatment (5.95 ± 0.88 ng/mL). To confirm drug washout, the RM-493 plasma levels were measured during the second treatment period: in the six participants who received RM-493 in the first period, RM-493 levels were 0.50 ± 0.20 ng/mL after 24h and undetectable (detection limit of assay < 0.50 ng/mL: personal communication from Rhythm) after 48-hour of placebo-treatment, ie, prior to the studies in the metabolic chamber.

Effects of RM-493 on resting energy expenditure (REE). All participants complied with the metabolic diet and standard exercise routine and maintained their body weight throughout the study. The primary endpoint, REE_C, was significantly increased by treatment with RM-493 (1856 ± 369 vs. 1745 ± 359 kcal/d with placebo, $P = .028$) (Figure 1), a treatment increase of 110 kcal/d (95% CI: 15 to 207 kcal/d). No period effect ($P = .32$) or treatment-period interaction ($P = .30$) was observed. REE_H was also measured as a secondary outcome after 72-hour of RM-493 administration. REE_H trended higher by 79 ± 130 kcal/d (95% CI: −4 to 162 kcal/d, $P = .059$) compared to placebo (Table 2).

Effects of RM-493 on total energy expenditure (TEE) and its components, respiratory quotient (RQ), and physical activity. The metabolic chamber assessments of 23-hour nonexercise, exercise, and sleeping EE and TEF did not differ between the treatment arms (Table 2). 23-hour nonexercise RQ was significantly lower during RM-493 treatment vs. placebo (0.833 ± 0.021 vs. 0.848 ± 0.022, $P = .02$) indicating a shift towards fat oxidation. Spontaneous

Table 1. Baseline clinical characteristics of 12 study subjects

Age, years	**34.9 ± 11.3**
Sex, F:M	6:6
Race, Black:Latino:White	9:1:2
Body Composition	
BMI, kg/m^2	
Females	38.0 ± 1.6
Males	33.4 ± 1.9
Lean body mass, kg	
Females	48.0 ± 5.6
Males	67.2 ± 8.5
% body fat	
Females	49.2 ± 1.5
Males	32.5 ± 6.8
Cardiovascular Parameters	
SBP, mmHg	115 ± 13
DBP, mmHg	62 ± 11
HR, bpm	64 ± 9.8
Metabolic Parameters	
Fasting glucose, mg/dL	91 ± 6
Hemoglobin A1C, %	5.5 ± 0.3
Fasting insulin, mcU/mL	18 ± 11
Fasting C-peptide, ng/mL	3.0 ± 1.2
QUICKI	0.32 ± 0.03
Lipids	
Total Cholesterol, mg/dL	163 ± 32
Calculated LDL, mg/dL	88 ± 32
HDL, mg/dL	51 ± 15
Triglycerides, mg/dL	139 ± 69
Free Fatty Acid, mEq/liter	0.454 ± 0.105

Values shown as means ± SD

Table 2. Energy expenditure, respiratory quotient, and spontaneous physical activity after placebo and RM-493 administration in obese individuals.

	RM-493	Placebo	P value
REE$_C$ (kcal/d)	1856 ± 369	1745 ± 359	0.028
REE$_H$ (kcal/d)	1849 ± 388	1769 ± 379	0.059
TEE (kcal/d)	2509 ± 420	2457 ± 399	0.089
Components of TEE			
Sleeping EE (kcal/m)	1.35 ± 0.19	1.35 ± 0.18	0.938
Exercise EE *(kcal/m)	4.49 ± 0.96	4.49 ± 1.05	0.998
TEF (kcal/m)	1.81 ± 0.31	1.76 ± 0.33	0.145
23-h RQ **	0.83 ± 0.02	0.85 ± 0.02	0.017
Spontaneous Physical Activity			
Hip counts / minute	304 ± 108	325 ± 85	0.300
Wrist counts / minute	1307 ± 335	1245 ± 465	0.434
Steps / minute	3.22 ± 1.32	3.51 ± 1.10	0.333
% Sedentary Time	80.7 ± 4.9	80.7 ± 4.6	0.940

Post-treatment values shown are unadjusted means ± SD Post-treatment values were adjusted for baseline value and treatment group using a mixed model regression. p values indicate significance for comparisons between RM-493 and placebo. * $n = 10$, two patients were excluded from analysis because of markedly different exercise efforts during the two phases.

** Averaged RQ over all non-exercise periods in the chamber.

and volitional physical activities as measured by hip and wrist accelerometers were similar in both treatment phases (Table 2).

Effects of RM-493 on metabolic parameters and hormonal profile. RM-493 treatment was associated with small increases in plasma fasting glucose, insulin, C-peptide, triglyceride, FFA, and total GLP-1 and PYY levels (Table 3). TSH levels were higher, albeit in the normal range following RM-493 administration, although there was no effect on total or free T3 and T4 levels (Table 3). Similarly, there were no differences in urinary cortisol adjusted for creat-

Table 3. Metabolic parameters and hormone levels after placebo and RM-493 administration in obese individuals.

	RM-493	Placebo	p value
Metabolic Parameters			
Fasting Glucose, mg/dL	95 ± 5.8	91 ± 3.6	0.003
Fasting Insulin, mcU/mL	26.2 ± 16	18.4 ± 12	0.008
Fasting C-peptide, ng/mL	3.6 ± 1.2	3.2 ± 1.2	0.043
Triglycerides, mg/dL	150 ± 70	129 ± 54	0.035
Free Fatty Acid, mEq/liter	0.442 ± 0.1	0.334 ± 0.1	0.009
Total GLP-1, pmol/liter	3.24 ± 1.15	2.27 ± 0.96	0.003
Total PYY, pg/ml	53.0 ± 19.1	38.7 ± 16.6	0.037
Thyroid Parameters			
TSH, mcIU/mL	1.86 ± 1.0	1.51 ± 0.73	0.009
T3, ng/dL	119 ± 40	112 ± 36	0.324
T4, mcg/dL	8.9 ± 1.3	8.7 ± 1.7	0.382
Free T3, pg/mL	2.6 ± 0.8	2.5 ± 0.7	0.312
Free T4, ng/dL	2.2 ± 0.4	2.1 ± 0.3	0.308

Post-treatment values shown are unadjusted means ± SD Post-treatment values were adjusted for baseline value and treatment group using a mixed model regression. P values indicate significance indicate significance for comparisons between RM-493- and placebo.

inine excretion and plasma levels of prolactin, ghrelin, irisin, and FGF-21 levels between the treatment groups (Supplemental Table 1).

Adverse effects associated with RM-493 and placebo administration. MC4R agonists have been previously reported to increase BP and heart rate (9, 14). No differences were observed in systolic (118 ± 10 vs. 118 ± 9 mm of Hg, $P = .69$) or diastolic (68 ± 8 vs. 69 ± 10 mmHg, $P = .56$) BP or in heart rate (67 ± 9 vs. 69 ± 11 bpm. $P = .11$) by 72-hour of treatment. During the RM-493 treatment phase, headache (n = 3), arthralgia (n = 2), nausea (n = 2), spontaneous penile erections (n = 1), female genital sensitivity (n = 2) were reported symptoms that were characterized as mild, transient, and resolved without sequelae. At the end of the study, 10 out of 12 participants correctly identified their RM-493 treatment order assignment.

Discussion

Safe pharmacotherapeutic options are needed to treat obesity. The role of the MC system in maintaining the balance between caloric intake and EE makes it an attractive target for therapeutic development (7, 8). We report the first clinical study of the effect of a MC4R agonist on EE. RM-493 administration increased REE measured in the metabolic chamber by 6.4% or 110 kcal/d. Consistent with these findings, RM-493 increased REE measured by hood indirect calorimetry, a less robust method, by 4.7% (~79 kcal/d, $P = .059$). These results are concordant with studies demonstrating increases of EE by melanocortin agonists in wild-type rodents (15, 16), but not in MC4R-deficient mice (17). The increase in REE is comparable to the increase in energy expenditure caused by single doses of sibutramine (18) or caffeine (19).

In our human study, RM-493 administration increased fat oxidation and circulating FFA levels. In rodents, MC4R agonists also increased fat oxidation, which was inhibited by the MCR antagonist, SHU9119 (15) and was lost in MC4R knockout mice (20). Note that in our study there was no confounding reduction in food intake to account for the lower RQ. Considering that low fatty acid oxidation is associated with increased risk of obesity (21), MC4R agonists may aid in weight loss by initiating a shift in substrate oxidation.

RM-493 (formerly BIM-22 493), is a cyclic peptide (Acetyl-L-arginyl-[L-cysteinyl-D-alanyl-L-histidinyl-D-phenylalanyl-L-arginyl-L-tryptophanyl-L-cysteine-]-amide) full agonist (EC_{50} = 0.27 nM) of human MC4R and binds with a Ki of 2.1 nM and ~10-fold selectivity over human MC3R (7). We administered RM-493 at 1 mg/24-hour, producing steady-state plasma concentrations of ~5 nM. Since RM-493 is not efficiently bound by plasma proteins, this concentration should activate MC4Rs, assuming it reaches the relevant tissue sites. Thus, the increase in REE by RM-493 is likely via agonism at MC4R.

In this small study, no significant effect of RM-493 on TEE, SEE, or TEF was found, possibly due to insufficient power. Further clinical studies are required to determine if the increase in REE is sustained and leads to weight loss in humans. However, in nonhuman primates, 8-week administration of RM-493 was associated with an increase (~14%) in total EE as measured by double-labeled water

and weight loss continued even after animals normalized food intake (9).

Chronic administration of RM-493 in Rhesus monkeys improved glucose tolerance and reduced serum triglycerides in the setting of weight loss (9). By contrast, we observed during short term treatment with stable caloric intake, RM-493 administration increased plasma triglyceride and FFA levels, likely via adipose tissue lipolysis. In rodents, central administration of MTII triggers lipid mobilization, increases sympathetic drive to white adipose tissue (22) and increases skeletal muscle AMP-activated protein kinase activity, which increases skeletal muscle FFA β-oxidation (23). The lower 23-hour nonexercise RQ following RM-493 administration in the current study reflects this increased fatty acid oxidation.

In contrast to the acute lowering of plasma insulin levels with MC4R agonists, in this study we observed statistically significant, but very small, increases in fasting plasma glucose, insulin, and C-peptide levels. The previously tested MC4R agonist LY2112688 (acetyl-D-arginyl-[L-cysteinyl-L-glutamyl-L-histidinyl-D-phenylalanyl-L-arginyl-L-tryptophanyl-L-cysteine]-amide) did not affect glucose and insulin levels (14). Consistent with this, in ongoing clinical studies of RM-493 (up to 12 weeks) no potentially adverse and/or significant changes in glucose, insulin and triglyceride parameters have been observed (unpublished results). Longer term clinical studies with RM-493 on insulin sensitivity are needed to put these observations in perspective. Similar to our findings, prior rodent studies show that central MCR stimulation can enhance hepatic glucose production, accompanied by a nonsignificant increase in fasting glucose levels (24). RM-493 treatment had no significant effect on urinary free cortisol levels or thyroid hormone levels. There was a small but significant increase in TSH levels, which may be explained by MC4R activation of hypothalamic TRH expression (25).

Recently, MC4R receptor activation in intestinal epithelial L-cells has been shown to acutely increase GLP-1 and PYY secretion in vivo in mice, and in colon explants from both mice and human (26). Similar to the rodent data, fasting total GLP-1 and PYY levels were slightly, but significantly increased during RM-493 administration in our study. Thus, it is possible that MC4R-induced GLP-1 production may contribute to eventual beneficial effects on insulin and glucose, while PYY (assumed to be PYY3–36) can positively impact energy metabolism during obesity treatment. It is possible that increases in both peptides may contribute to the weight loss effect of RM-493. Given the small number of patients evaluated, these studies require follow up in larger populations.

Unlike LY2112688, short term administration of RM-493 was not associated with increased BP and heart rate (14). In an acute nonhuman primate study, both LY2112688 and RM-493 reduced food intake, while only LY2112688 increased heart rate and BP (9). The current study and additional phase 1 studies in which RM-493 was dosed for up to 28 days (unpublished results), did not elicit adverse cardiovascular effects. In our study, nausea, penile erections, and female genital sensitivity were observed, similar to other studies of melanocortin agonists in humans (14, 27–29). Larger, long term clinical trials will be required to evaluate overall safety and tolerability of RM-493 for the treatment of obesity, including cardiovascular effects.

Our study has several limitations. First, the treatment duration in this proof-of-concept study was 72 hours; longer studies are needed to see if the REE increase and changes in other analytes is sustained. Second, the study was small, powered to detect changes in REEmeasured in a sensitive metabolic chamber, and was not powered to detect changes in TEE, SEE, or TEF. Third, 75% of study participants were African American. Although, there is no evidence to suggest racial or ethnic differences in response to MC4R agonists, studies with a more diverse population would increase the generality of our findings. Fourth, while not detected, we cannot completely rule out the possibility of carry-over effects, since patients entered the second crossover period without an extended washout.

In summary, we report that a three-day administration of a MC4R agonist, RM-493, significantly increased REE and preferentially increased fat oxidation in obese individuals. RM-493 exhibited minimal side effects and no pressor effects observed with prior MC4R agonists in development. Our study provides a mechanistic rationale for the use of selective MC4R agonists, in the treatment of obesity and the metabolic syndrome. Longer-term studies in obese individuals are necessary to determine whether MC4R agonists are effective therapeutic agents for weight loss.

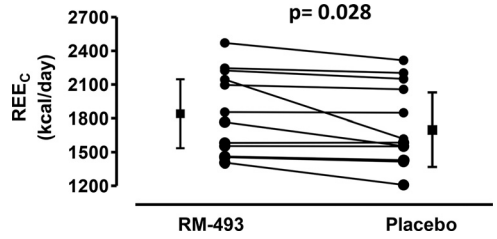

Figure 2. Effect of RM-493 vs placebo on resting energy expenditure (REE_C) at the end of treatment period. REE_C was measured on the third treatment day of each treatment arm in the metabolic chamber at thermoneutrality in the resting, postabsorptive state as the mean of two 30-minute rest periods. Individual data points and aggregate data with error bars are overall mean ± 95% CI. P values indicate significance of treatment differences using a paired *t* test.

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K.Y.C participated in the design of the study, collection of data, data analysis, writing of the manuscript, and approved the final manuscript. R.M. participated in the collection of data, data analysis, writing of the manuscript and approved the final manuscript. B.S.A, K.P.M, P.S., R.J.B., and T.P participated in the conduct of the study, collection of data, and approved the final manuscript. X.Z. and M.R. participated in data management, analysis, and approved the final manuscript. R.D.C and B.L.P participated in the study design, interpretation of data, writing of the manuscript, and approved the final manuscript. M.C.S., K.G., L.V.P, and M.L.R participated in the design, interpretation of data, writing of the manuscript, and approved the final manuscript. M.C.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Disclosure Summary: Keith Gottesdiener and Lex Van der Ploeg are employees of Rhythm Pharmaceuticals, a privately held Biotechnology company which is developing RM-493 for the treatment of obesity.

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References

1. **Cone RD.** The Central Melanocortin System and Energy Homeostasis. *Trends Endocrinol Metab*. 1999;10:211–216.
2. **Cone RD.** Studies on the physiological functions of the melanocortin system. *Endocr Rev*. 2006;27:736–749.
3. **Fan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD.** Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature*. 1997;385:165–168.
4. **Vaisse C, Clement K, Durand E, Hercberg S, Guy-Grand B, Froguel P.** Melanocortin-4 receptor mutations are a frequent and heterogeneous cause of morbid obesity. *J Clin Invest*. 2000;106:253–262.
5. **Krakoff J, Ma L, Kobes S, Knowler WC, Hanson RL, Bogardus C, Baier LJ.** Lower metabolic rate in individuals heterozygous for either a frameshift or a functional missense MC4R variant. *Diabetes*. 2008;57:3267–3272.
6. **Hinney A, Volckmar AL, Knoll N.** Melanocortin-4 receptor in energy homeostasis and obesity pathogenesis. *Prog Mol Biol Transl Sci*. 2013;114:147–191.
7. **Fani L, Bak S, Delhanty P, van Rossum EF, van den Akker EL.** The melanocortin-4 receptor as target for obesity treatment: a systematic review of emerging pharmacological therapeutic options. *Int J Obes (Lond)*. 2014;38:163–169.
8. **Tao YX.** The melanocortin-4 receptor: physiology, pharmacology, and pathophysiology. *Endocr Rev*. 2010;31:506–543.
9. **Kievit P, Halem H, Marks DL, Dong JZ, Glavas MM, Sinnayah P, Pranger L, Cowley MA, Grove KL, Culler MD.** Chronic treatment with a melanocortin-4 receptor agonist causes weight loss, reduces insulin resistance, and improves cardiovascular function in diet-induced obese rhesus macaques. *Diabetes*. 2013;62:490–497.
10. **Schutz Y, Jequier E.** 1998 Resting energy expenditure, thermic effect of food and total energy expenditure. New York, NY: Marcel Dekker
11. **Rumpler WV, Seale JL, Conway JM, Moe PW.** Repeatability of 24-h energy expenditure measurements in humans by indirect calorimetry. *Am J Clin Nutr*. 1990;51:147–152.
12. **Ravussin E, Lillioja S, Anderson TE, Christin L, Bogardus C.** Determinants of 24-hour energy expenditure in man. Methods and results using a respiratory chamber. *J Clin Invest*. 1986;78:1568–1578.
13. **Cooper JA, Watras AC, O'Brien MJ, Luke A, Dobratz JR, Earthman CP, Schoeller DA.** Assessing validity and reliability of resting metabolic rate in six gas analysis systems. *J Am Diet Assoc*. 2009;109:128–132.
14. **Greenfield JR, Miller JW, Keogh JM, Henning E, Satterwhite JH, Cameron GS, Astruc B, Mayer JP, Brage S, See TC, Lomas DJ, O'Rahilly S, Farooqi IS.** Modulation of blood pressure by central melanocortinergic pathways. *N Engl J Med*. 2009;360:44–52.
15. **Hwa JJ, Ghibaudi L, Gao J, Parker EM.** Central melanocortin system modulates energy intake and expenditure of obese and lean Zucker rats. *Am J Physiol Regul Integr Comp Physiol*. 2001;281:R444–451.
16. **Cowley MA, Pronchuk N, Fan W, Dinulescu DM, Colmers WF, Cone RD.** Integration of NPY, *AGRP, and melanocortin signals in the hypothalamic paraventricular nucleus: evidence of a cellular basis for the adipostat*. *Neuron*. 1999;24:155–163.
17. **Hsiung HM, Hertel J, Zhang XY, Smith DP, Smiley DL, Heiman ML, Yang DD, Husain S, Mayer JP, Zhang L, Mo H, Yan LZ.** A novel and selective beta-melanocyte-stimulating hormone-derived peptide agonist for melanocortin 4 receptor potently decreased food intake and body weight gain in diet-induced obese rats. *Endocrinology*. 2005;146:5257–5266.
18. **Hansen DL, Toubro S, Stock MJ, Macdonald IA, Astrup A.** Thermogenic effects of sibutramine in humans. *Am J Clin Nutr*. 1998;68:1180–1186.
19. **Astrup A, Toubro S, Cannon S, Hein P, Breum L, Madsen J.** Caffeine: a double-blind, placebo-controlled study of its thermogenic, metabolic, and cardiovascular effects in healthy volunteers. *Am J Clin Nutr*. 1990;51:759–767.
20. **Butler AA, Marks DL, Fan W, Kuhn CM, Bartolome M, Cone RD.** Melanocortin-4 receptor is required for acute homeostatic responses to increased dietary fat. *Nat Neurosci*. 2001;4:605–611.
21. **Zurlo F, Lillioja S, Esposito-Del Puente A, Nyomba BL, Raz I, Saad MF, Swinburn BA, Knowler WC, Bogardus C, Ravussin E.** Low ratio of fat to carbohydrate oxidation as predictor of weight gain: study of 24-h RQ. *Am J Physiol*. 1990;259:E650–657.
22. **Fan W, Dinulescu DM, Butler AA, Zhou J, Marks DL, Cone RD.** The central melanocortin system can directly regulate serum insulin levels. *Endocrinology*. 2000;141:3072–3079.
23. **Tanaka T, Masuzaki H, Yasue S, Ebihara K, Shiuchi T, Ishii T, Arai N, Hirata M, Yamamoto H, Hayashi T, Hosoda K, Minokoshi Y, Nakao K.** Central melanocortin signaling restores skeletal muscle

AMP-activated protein kinase phosphorylation in mice fed a high-fat diet. *Cell Metab*. 2007;5:395–402.

24. **Heijboer AC, van den Hoek AM, Pijl H, Voshol PJ, Havekes LM, Romijn JA, Corssmit EP.** Intracerebroventricular administration of melanotan II increases insulin sensitivity of glucose disposal in mice. *Diabetologia*. 2005;48:1621–1626.
25. **Harris M, Aschkenasi C, Elias CF, Chandrankunnel A, Nillni EA, Bjoorbaek C, Elmquist JK, Flier JS, Hollenberg AN.** Transcriptional regulation of the thyrotropin-releasing hormone gene by leptin and melanocortin signaling. *J Clin Invest*. 2001;107:111–120.
26. **Panaro BL TI, Engelstoft MS, Matthews RT, Digby GJ, Møller CL, Svendsen B, Gribble F, Reimann F, Holst JJ, Holst B, Schwartz TW, Cox HM, Cone RD.** 2014 The Melanocortin-4 Receptor is Expressed in Enteroendocrine L Cells and Regulates the Release of Peptide YY and Glucagon-Like Peptide. Cell Metabolism (In press)
27. **Royalty JE, Konradsen G, Eskerod O, Wulff BS, Hansen BS.** Investigation of safety, tolerability, pharmacokinetics, and pharmacodynamics of single and multiple doses of a long-acting alpha-MSH analog in healthy overweight and obese subjects. *J Clin Pharmacol*. 2014;54:394–404.
28. **Safarinejad MR.** Evaluation of the safety and efficacy of bremelanotide, a melanocortin receptor agonist, in female subjects with arousal disorder: a double-blind placebo-controlled, fixed dose, randomized study. *J Sex Med*. 2008;5:887–897.
29. **Diamond LE, Earle DC, Rosen RC, Willett MS, Molinoff PB.** Double-blind, placebo-controlled evaluation of the safety, pharmacokinetic properties and pharmacodynamic effects of intranasal PT-141, a melanocortin receptor agonist, in healthy males and patients with mild-to-moderate erectile dysfunction. *Int J Impot Res*. 2004;16:51–59.