CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Elsevier - Publisher Connector

Cell Metabolism
Article

Activation of Human Brown Adipose Tissue by a β3-Adrenergic Receptor Agonist

Aaron M. Cypess,[1,*] Lauren S. Weiner,[1] Carla Roberts-Toler,[1] Elisa Franquet Elía,[2] Skyler H. Kessler,[1] Peter A. Kahn,[3] Jeffrey English,[2] Kelly Chatman,[4] Sunia A. Trauger,[4] Alessandro Doria,[5] and Gerald M. Kolodny[2]
[1]Section of Integrative Physiology and Metabolism, Research Division, Joslin Diabetes Center, Harvard Medical School, Boston, MA 02215, USA
[2]Division of Nuclear Medicine and Molecular Imaging, Department of Radiology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA
[3]Albert Einstein College of Medicine, Bronx, NY 10461, USA
[4]Small Molecule Mass Spectrometry Facility, FAS Division of Science, Harvard University, Cambridge, MA 02138, USA
[5]Section of Genetics and Epidemiology, Research Division, Joslin Diabetes Center, Harvard Medical School, Boston, MA 02215, USA
*Correspondence: Aaron.Cypess@joslin.harvard.edu, aaron.cypess@nih.gov
http://dx.doi.org/10.1016/j.cmet.2014.12.009

SUMMARY

Increasing energy expenditure through activation of endogenous brown adipose tissue (BAT) is a potential approach to treat obesity and diabetes. The class of β3-adrenergic receptor (AR) agonists stimulates rodent BAT, but this activity has never been demonstrated in humans. Here we determined the ability of 200 mg oral mirabegron (Myrbetriq, Astellas Pharma, Inc.), a β3-AR agonist currently approved to treat overactive bladder, to stimulate BAT as compared to placebo. Mirabegron led to higher BAT metabolic activity as measured via ^{18}F-fluorodeoxyglucose (^{18}F-FDG) using positron emission tomography (PET) combined with computed tomography (CT) in all twelve healthy male subjects (p = 0.001), and it increased resting metabolic rate (RMR) by 203 ± 40 kcal/day (+13%; p = 0.001). BAT metabolic activity was also a significant predictor of the changes in RMR (p = 0.006). Therefore, a β3-AR agonist can stimulate human BAT thermogenesis and may be a promising treatment for metabolic disease.

INTRODUCTION

Obesity and metabolic disease result when energy intake consistently exceeds energy expenditure. One appealing new target for treatment is the activation of brown adipose tissue (BAT), an organ recently found to be functional in adult humans (van Marken Lichtenbelt et al., 2009; Cypess et al., 2009; Virtanen et al., 2009; Zingaretti et al., 2009; Saito et al., 2009). Cold exposure causes the sympathetic nervous system to release norepinephrine and induce human BAT thermogenesis through consumption of fatty acids and glucose. In addition to improving energy balance, rodent models have shown that chronic stimulation of BAT leads to improved glucose tolerance and the release of adipokines that beneficially regulate metabolism (Bartelt et al., 2011; Hondares et al., 2011). Unfortunately, nonspecific sympathomimetic drugs cannot stimulate human BAT without marked effects on the cardiovascular system (Cypess et al., 2012; Vosselman et al., 2012; Carey et al., 2013).

One area of focus for over two decades has been the stimulation of BAT energy expenditure and nutrient consumption through activation of the β3-adrenergic receptor (AR), which is expressed in humans on the surfaces of brown and white adipocytes, urinary bladder, and potentially other tissues (Virtanen et al., 2009; Cypess et al., 2013; Ursino et al., 2009). Previous phase 2 clinical trials with members of this class demonstrated improved glucose disposal, decreased plasma triglycerides, and increased RMR (Cawthorne et al., 1992; Weyer et al., 1998; Larsen et al., 2002; Redman et al., 2007). Nevertheless, none of these drugs ultimately achieved regulatory approval for the treatment of obesity or metabolic disease. Reasons for this lack of success have been attributed to multiple factors, including poor oral bioavailability and substantial cross-reactivity with the β1-AR that caused undesirable cardiovascular effects (Arch, 2011). More fundamentally, it was unknown if the β3-AR agonists could stimulate human BAT. This issue is still unresolved, because these drugs were evaluated prior to the discoveries of functional human BAT and how to measure its metabolic activity via prospective ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET/CT imaging (van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009; Muzik et al., 2013; Yoneshiro et al., 2013; van der Lans et al., 2013). Recently, the new β3-AR agonist mirabegron was approved for treatment of overactive bladder. In addition to having satisfactory bioavailability (Malik et al., 2012), mirabegron has a higher in vitro binding affinity for the human β3-AR compared to other members of its class (Takasu et al., 2007), as well as the mixed sympathomimetic ephedrine (Vansal and Feller, 1999), making it a promising candidate.

RESULTS AND DISCUSSION

Effects of Acute Cold Exposure on BAT, RMR, and Vital Signs

To test the ability of mirabegron to acutely stimulate human BAT, we wanted to evaluate its efficacy in subjects who were already known to have detectable BAT, thereby reducing the likelihood of a false-negative finding (Cypess et al., 2012; Carey et al., 2013). Fifteen eligible subjects were screened first with cold

Table 1. Clinical Characteristics of Subjects

Characteristic[a] (Units)	Value[b]
Age (years)	22.2 ± 0.6
Height (cm)	177 ± 2
Weight (kg)	71.3 ± 2.4
Body-mass Index (kg/m^2)	22.7 ± 0.5
Waist-hip Ratio	0.85 ± 0.01
Body Fat[c] (kg)	13.4 ± 0.8
Fat-free Mass[c] (kg)	58.6 ± 2.7
Systolic Blood Pressure (mmHg)	112 ± 2
Diastolic Blood Pressure (mmHg)	68 ± 2
Heart Rate (bpm)	57 ± 2
Resting Metabolic Rate (kcal/day)	1,573 ± 56

See also Figure S1.
[a]Baseline values determined prior to the dosing of mirabegron.
[b]Values are mean ± SEM.
[c]Measured on average 2 weeks prior to the dosing of mirabegron.

exposure, and twelve had detectable BAT (Supplemental Experimental Procedures; Figure S1A available online). The amount of BAT activity, based on the standard uptake value (SUV), was comparable to that reported in other studies using lower doses of radiotracer (Vosselman et al., 2012). In these twelve subjects (Table 1), cold exposure increased RMR (128 ± 32 kcal/day, or +8%) (p = 0.001), systolic blood pressure (BP), and diastolic BP; decreased heart rate (Figures S1B–S1E); and led to metabolite concentrations consistent with what we have previously reported (Cypess et al., 2012). Safety considerations limited the subjects to three PET/CT scans each, so we could evaluate only one dose of mirabegron in comparison to placebo. We selected 200 mg since this dose has a higher efficacy than the currently approved dose of 50 mg for reducing the symptoms of overactive bladder and was therefore more likely to have a detectable effect on BAT metabolic activity. In addition, the 200 mg dosage has been well-tolerated even after 12 weeks of daily oral administration (Chapple et al., 2013).

Effects of Mirabegron on Tissue Glucose Uptake, RMR, Vital Signs, and Plasma Metabolites

Compared to placebo, for all twelve subjects BAT glucose uptake was significantly higher after treatment with mirabegron (median 132, interquartile range 70–253 ml·SUVmean·g/ml, p = 0.001) (Figures 1A, 1B, and S2). The principal sites of detectable BAT were the cervical-supraclavicular-axillary adipose tissue depots, but in some subjects, glucose uptake was seen even in the paraspinal, periaortic, perihepatic, perirenal, and perisplenic regions. The correlation between drug- and cold-induced BAT glucose uptake was not significant (Figure S3), indicating that it is not consistently reliable to use only one method of stimulation—either cold or mirabegron—to accurately measure whole-body BAT mass or activity.

Compared to placebo, mirabegron significantly increased RMR (203 ± 40 kcal/d, or +13%) (p = 0.001), HR (14 ± 3 bpm) (p = 0.002), and systolic BP (11 ± 2 mmHg) (p = 0.002), but not diastolic BP (2 ± 1 mmHg) (p = 0.07) (Figures 1C–1F). This drug-induced stimulation of the cardiovascular system was considerably lower than what has been reported for broadly acting sympathomimetics such as ephedrine or isoproterenol, particularly for the extent of changes seen in RMR (Cypess et al., 2012; Vosselman et al., 2012; Carey et al., 2013). There were no unanticipated adverse effects.

Metabolites were drawn 3.5 hr after dosing of mirabegron and placebo, which was the reported T_{max} (Malik et al., 2012). Mirabegron was detectable in the plasma with a mean concentration of 310 ± 73 ng/mL (p = 0.001), values comparable to other studies (Malik et al., 2012). Administration of mirabegron was notable for inducing higher levels of glucose, nonesterified fatty acids (NEFAs), β-hydroxybutyrate, insulin, C-peptide, and significant elevations in HOMA-IR (p = 0.002) (Table S1). Maximal glucose uptake (SUVmax) was lower in the myocardium, though it did not achieve significance after applying Bonferroni's correction. No apparent differences were seen in the subcutaneous white adipose tissue (WAT), skeletal muscle, or liver (Figures 2A–2D).

Predictors of Drug-Induced Changes in RMR

Since both BAT and WAT express the β3-AR (Virtanen et al., 2009), as exploratory analyses, we compared the changes in RMR with BAT activity and WAT mass. We found a close association between change in RMR and BAT activity stimulated by mirabegron (p = 0.006) but not placebo (p = 0.17) (Figures 3A and 3B). There was also a positive relationship between changes in RMR and body fat mass in response to mirabegron (p = 0.02) but not placebo (p = 0.64) (Figures 3C and 3D). In contrast, there were no associations between the change in RMR and the change in heart rate (R^2 = 0.00, p = 0.97), systolic BP (R^2 = 0.01, p = 0.14), or fat-free mass (R^2 = 0.00, p = 0.96).

A challenge to studying human thermogenesis is determining the contribution of each organ. Mirabegron increased BAT glucose uptake, which correlates directly with tissue thermogenesis (Ouellet et al., 2012), and the degree of activation accounted for 50% of the variability in drug-induced changes in RMR. The amount of BAT thermogenesis in absolute terms must still be determined, particularly as there may be an important contribution from nonselective activation of other β-AR's on other tissues. Nevertheless, our findings indicate that human BAT may play a role in the thermogenesis associated with β3-AR agonist treatment as well as exposure to mild cold (Chen et al., 2013). An intriguing issue is the proportional contribution by the two different brown adipocyte lineages: the constitutive "brown" adipocytes in the cervical and supraclavicular depots and the recruitable "beige/brite" adipocytes in the supraclavicular, abdominal, and other sites (Wu et al., 2012; Sharp et al., 2012; Lidell et al., 2013; Cypess et al., 2013; Jespersen et al., 2013; Sacks et al., 2013). Based on the wide distribution of detectable glucose uptake, it is likely that mirabegron stimulates both kinds of BAT, though more detailed in vitro studies are required to quantify what functional differences may exist between these adipocytes.

Potential Therapeutic Applications of Chronic Treatment with β3-AR Agonists

An important question remains as to what clinical benefit there could be from stimulation of human BAT with β3-AR agonists. Attention has been given to weight loss through increased energy expenditure as a treatment for obesity. Previous clinical

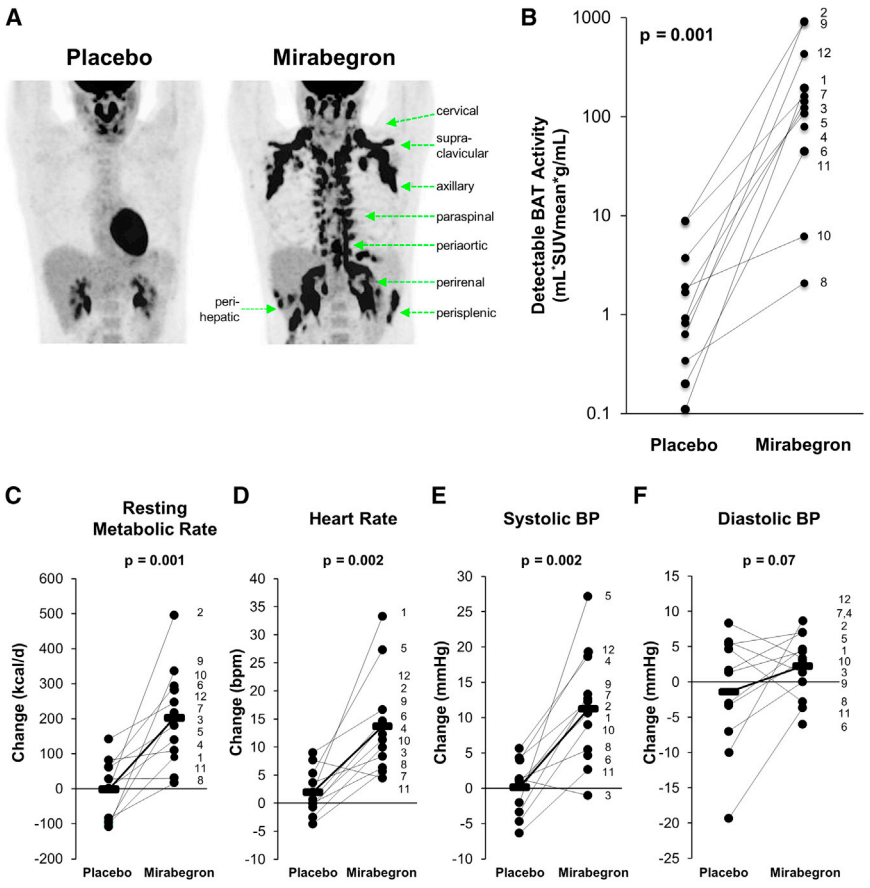

Figure 1. Metabolic Effects of the β3-AR Agonist Mirabegron

(A) PET images of a 21-year-old man who was given placebo (left) or 200 mg of the β3-AR agonist mirabegron (right). Twelve male subjects were given placebo or 200 mg mirabegron.

(B) BAT metabolic activity as reflected by ^{18}F-FDG uptake.

(C) Resting metabolic rate.

(D) Heart rate.

(E) Systolic BP.

(F) Diastolic BP.

Each circle represents a single subject, and the numbers correspond to subject identification number in Figure S2. The dashes represent the mean. See also Figure S2.

trials of β3-AR agonists did not achieve weight loss within at most 8 weeks of treatment. This may have been in part because the doses used were insufficient to activate BAT thermogenesis (Weyer et al., 1998; Larsen et al., 2002; Redman et al., 2007). At the 200 mg dose used in the current study, the observed maximal increase in energy expenditure would lead to an eventual weight loss of ~5 kg in the first year and 10 kg by the end of 3 years (Hall et al., 2011). Though promising, the actual amount of weight loss would likely be less, since 200 kcal/day was the peak energy expenditure and not sustained throughout the day.

However, it is important to consider that in rodent models, treatment with β3-AR agonists chronically can improve glucose disposal and increase RMR before a reduction in body weight is seen (de Souza et al., 1997). This distinction between the acute and chronic effects of treatment with mirabegron applies to the current study. In the acute setting, we saw a significant increase in HOMA-IR, a measure of insulin resistance. As with the changes in heart rate, it is not known if this effect is from off-target binding to other β-AR's, and future studies are needed to determine which metabolic changes are attributable specifically to β3-AR agonists. In the present study, in addition to the effects on BAT, there was evidence for β3-AR agonist stimulation of WAT lipolysis (Kim et al., 2006), which was reflected by the non-significantly higher levels of serum NEFAs and lower myocardial glucose uptake that is associated with this change in fuel availability (Vosselman et al., 2012). Thus, chronic treatment with a β3-AR agonist in humans may improve multiple facets of metabolism even in the absence of weight loss through consumption of lipids and glucose and also the release of beneficial adipokines (Stanford et al., 2013; Villarroya et al., 2013).

This study is limited by its small size and duration. Also, the young, lean, healthy male subjects with detectable BAT were selected deliberately for the purpose of a proof-of-concept study, so the efficacy of mirabegron needs to be evaluated in women and other populations, such as those with different ages and BMIs. In addition, the changes in RMR and metabolism were measured after administration of a single dose of mirabegron, so we do not know if chronic treatment would yield the metabolic benefits seen with other β3-AR agonists. Finally, the decision to use the more potent 200 mg dose of mirabegron was advantageous in that all twelve subjects had detectable BAT activity that was higher than placebo. For this small sample size, the wide range of BAT activities we observed was crucial for learning meaningful aspects about human BAT physiology. However, the 200 mg dose was also higher than necessary to selectively activate only the β3-AR (Takasu et al., 2007; FDA 2012a). As a result, we observed binding to the β1-AR with resultant tachycardia, a treatment-emergent adverse event noted in several clinical trials of mirabegron (Malik et al., 2012; Sacco and Bientinesi, 2012). Given the acute stimulation of the cardiovascular system, the long-term safety of this approach must be established. It remains to be determined if the approved daily dose of 50 mg for overactive bladder, which was associated with smaller effects on heart rate (FDA 2012b), will sufficiently stimulate BAT growth and thermogenesis. Given that activation of the myocardial β3-AR has limited chronotropic effects (Masutani et al., 2013), concomitant administration of 200 mg mirabegron with a β1-AR blocker could mitigate the undesirable cardiovascular stimulation. Alternatively, it is possible that the off-target effects may be satisfactorily reduced by newly designed members of the class having greater selectivity for the β3-AR.

In summary, we demonstrate that the β3-AR agonist mirabegron acutely stimulates human BAT thermogenesis and

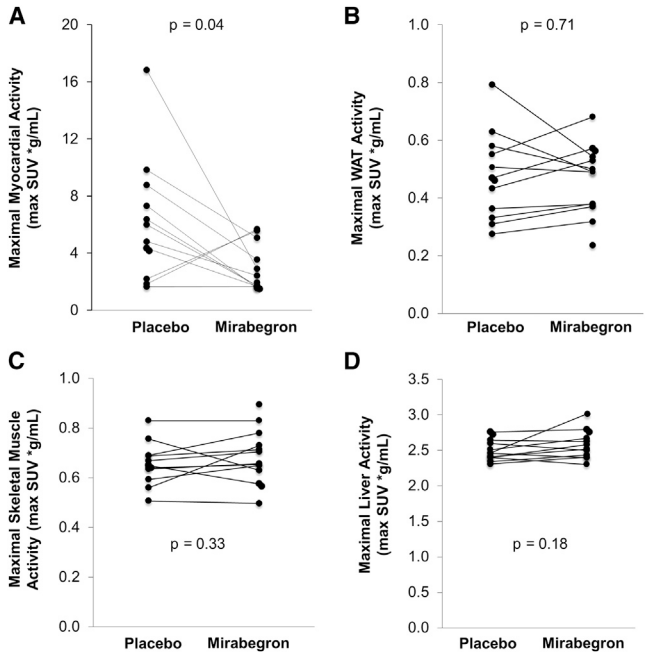

Figure 2. Tissue Glucose Uptake

^{18}F-FDG uptake in the twelve volunteers when given placebo or 200 mg mirabegron is shown for different tissues. Each circle represents a single subject.

(A) Myocardium.

(B) Subcutaneous WAT.

(C) Skeletal muscle.

(D) Liver.

See also Table S1.

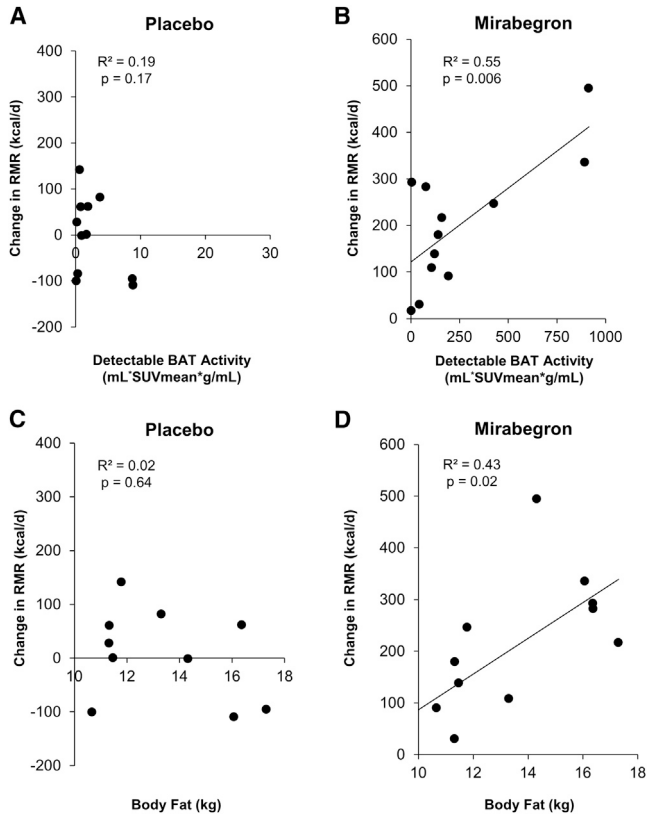

Figure 3. Predictors of Resting Metabolic Rate

(A) Placebo-induced BAT activity.

(B) Two-hundred milligram mirabegron-induced BAT activity.

(C) Body fat mass with placebo.

(D) Body fat mass with 200 mg mirabegron.

See also Figure S3.

increases RMR. Given that mirabegron is already approved for treatment of overactive bladder, we anticipate that these findings will accelerate the development of pharmacological strategies designed to increase energy expenditure and treat obesity and metabolic disease.

EXPERIMENTAL PROCEDURES

Additional information can be found in Supplemental Experimental Procedures.

Study Approval

This clinical trial is registered with ClinicalTrials.gov (NCT01783470) and has the FDA Investigational New Drug (IND) registration number 116246. It was approved by the Human Studies Institutional Review Boards of Beth Israel Deaconess Medical Center (BIDMC) and Joslin Diabetes Center (JDC). Healthy volunteers were recruited through electronic advertisements and provided written informed consent.

Study Day

Fifteen subjects underwent cold-activated ^{18}F-FDG PET/CT scanning, consisting of wearing a vest with circulating water set to 14°C for 120 min, as described in Cypess et al. (2012), and twelve had detectable BAT. These twelve subjects then participated in two more imaging days in which they were given 200 mg per os mirabegron or placebo control in randomized order that was unblinded. To reduce the contribution of cold-activated BAT, for the placebo and mirabegron study days, we maintained the room temperature above 23°C throughout the entire study visit. There was a washout period of at least 48 hr between each of the three interventions. Given the plasticity of BAT activity in response to seasonal changes in outdoor temperature, we restricted the average interval between the subjects' first and last study to 28.1 ± 7.4 days. Vital signs, body composition, and metabolites were measured as described previously (Cypess et al., 2012).

Measurement of Plasma Mirabegron Concentration

All reagents were HPLC grade. For an internal standard, a 1,000 pg/μL stock solution of [$^{13}C_6$]-Mirabegron (Alsachim) was dissolved in methanol (Fisher Scientific). A total of 20 μl (20,000 pg) of this standard was added to 500 μl of the subjects' plasma. For an ion-pairing agent, we added 400 μl of 20 mM ammonium acetate (Sigma). Liquid-liquid extraction of mirabegron and the standard was performed using 3 ml diethyl-ether (Sigma), and the solution was mixed at room temperature for 10 min. The phases were separated through centrifugation for 5 min at 2,361 × *g* at room temperature. The upper organic phase was isolated and dried under a stream of nitrogen at room temperature. The precipitate was resuspended in 1.75 ml methanol and then dried under vacuum at room temperature.

LC-MS/MS Analyses of Mirabegron

An Agilent 6460 LC-MS/MS triple quadrupole mass spectrometer, coupled to a 1290 uHPLC and atmospheric chemical ionization (APCI), was used for the detection and quantitation of mirabegron in positive ion mode using a multiple reaction monitoring approach. An isotopologue of mirabegron [$^{13}C_6$] was used an internal standard. More comprehensive experimental details are provided in the Supplemental Experimental Procedures.

Sample Size Calculation

The primary hypothesis was that BAT activity following a single dose of 200 mg mirabegron would be significantly different from that following placebo. BAT

activity was considered as a continuous variable, and each subject was his own paired control. Since we were conducting a pilot study with no prior data on the effects of mirabegron on human BAT, we used existing data from our prior studies of cold-exposed men to determine the detectable difference between placebo and mirabegron based on a predetermined sample size. Additional details can be found in the Supplemental Experimental Procedures.

Statistical Analysis

We analyzed the data using JMP Pro 9.0.0 software (SAS Institute, Inc.). Since the sample size of twelve subjects limited the ability to demonstrate that measurements were normally distributed, we used the nonparametric Wilcoxon sign-ranks test to assess the primary and secondary endpoints. All p values presented are two tailed. p values ≤ 0.05 were considered to indicate statistical significance for the primary outcome. For the secondary endpoints, p values ≤ 0.002 were considered significant based on a Bonferroni correction for 21 variables (RMR, systolic BP, diastolic BP, and HR; glucose uptake in myocardium, WAT, skeletal muscle, and liver; and blood concentrations of glucose, NEFAs, lactic acid, β-hydroxybutyrate, insulin, C-peptide, HOMA-IR, glucagon, norepinephrine, cortisol, TSH, free T4, and total T3). Drug- and cold-induced changes in BAT glucose uptake were correlated using Spearman's ρ. The study had >80% power ($\alpha = 0.05$) to detect a difference in the primary outcome between mirabegron and placebo treatment equal to 20% of the effect of cold.

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures, one table, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2014.12.009.

AUTHOR CONTRIBUTIONS

A.M.C., L.S.W., J.E., and G.M.K. designed the experiment. A.M.C., L.S.W., C.R.-T., P.A.K., K.C., S.T., A.D., and G.M.K. wrote the manuscript. A.M.C., L.S.W., E.F.E., S.H.K., P.A.K., and G.M.K. were responsible for quantification of human brown adipose tissue function. C.R.-T., K.C., and S.T. were responsible for measurement of plasma mirabegron concentrations. A.M.C., S.H.K., and A.D. carried out the statistical analysis. All authors contributed to editing the manuscript and had final approval of the submitted manuscript.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health (NIH) grants K23 DK081604 and P30 DK036836 (including a P&F award) and the Intramural Research Program of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK); the Fundación Alfonso Martín Escudero; Harvard Catalyst | The Harvard Clinical and Translational Science Center (National Center for Research Resources award UL1 RR025758 and National Center for Advancing Translational Science awards UL1TR000170 and UL1TR001102) and financial contributions from Harvard University and its affiliated academic healthcare centers. The content is solely the responsibility of the authors and does not necessarily represent the official views of Harvard Catalyst, Harvard University and its affiliated academic healthcare centers, or the NIH.

We thank the excellent support provided by Theresa Jundzil and the BIDMC Clinical Research Center nursing team, Bionutrition Core, research pharmacy, and nuclear medicine technologists; Murray Mittleman, Hillary Keenan, and Elissa Wilker for their biostatistical advice; C. Ronald Kahn and Yu-Hua Tseng for their input in preparing this manuscript; Kevin Hall for his discussions about energy balance; Marc Reitman for his discussions about clinical pharmacology; Nancy Brown and Angela Lavoie for their regulatory guidance; and Olivia Lovegreen and Liana Coviello for their administrative support. Finally, we are grateful to our volunteers for their commitment to the studies.

Dr. Cypess is a recipient of sponsored research grants from Chugai Pharmaceutical Co., Ltd, and Molecular Metabolism, LLC, both through Joslin Diabetes Center; and he has received payment for lecturing about clinical diabetes on behalf of Joslin Diabetes Center to employees of Sanofi, Genentech, Eli Lilly, Janssen, and Regeneron. Dr. Doria has received research support from Sanofi.

Received: August 14, 2014
Revised: October 2, 2014
Accepted: December 15, 2014
Published: January 6, 2015

REFERENCES

Arch, J.R. (2011). Challenges in β(3)-Adrenoceptor Agonist Drug Development. Ther. Adv. Endocrinol. Metab. *2*, 59–64.

Bartelt, A., Bruns, O.T., Reimer, R., Hohenberg, H., Ittrich, H., Peldschus, K., Kaul, M.G., Tromsdorf, U.I., Weller, H., Waurisch, C., et al. (2011). Brown adipose tissue activity controls triglyceride clearance. Nat. Med. *17*, 200–205.

Carey, A.L., Formosa, M.F., Van Every, B., Bertovic, D., Eikelis, N., Lambert, G.W., Kalff, V., Duffy, S.J., Cherk, M.H., and Kingwell, B.A. (2013). Ephedrine activates brown adipose tissue in lean but not obese humans. Diabetologia *56*, 147–155.

Cawthorne, M.A., Sennitt, M.V., Arch, J.R., and Smith, S.A. (1992). BRL 35135, a potent and selective atypical beta-adrenoceptor agonist. Am. J. Clin. Nutr. *55* (*Suppl*), 252S–257S.

Chapple, C.R., Dvorak, V., Radziszewski, P., Van Kerrebroeck, P., Wyndaele, J.J., Bosman, B., Boerrigter, P., Drogendijk, T., Ridder, A., Van Der Putten-Slob, I., and Yamaguchi, O.; Dragon Investigator Group (2013). A phase II dose-ranging study of mirabegron in patients with overactive bladder. Int. Urogynecol. J. *24*, 1447–1458.

Chen, K.Y., Brychta, R.J., Linderman, J.D., Smith, S., Courville, A., Dieckmann, W., Herscovitch, P., Millo, C.M., Remaley, A., Lee, P., and Celi, F.S. (2013). Brown fat activation mediates cold-induced thermogenesis in adult humans in response to a mild decrease in ambient temperature. J. Clin. Endocrinol. Metab. *98*, E1218–E1223.

Cypess, A.M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A.B., Kuo, F.C., Palmer, E.L., Tseng, Y.H., Doria, A., et al. (2009). Identification and importance of brown adipose tissue in adult humans. N. Engl. J. Med. *360*, 1509–1517.

Cypess, A.M., Chen, Y.C., Sze, C., Wang, K., English, J., Chan, O., Holman, A.R., Tal, I., Palmer, M.R., Kolodny, G.M., and Kahn, C.R. (2012). Cold but not sympathomimetics activates human brown adipose tissue in vivo. Proc. Natl. Acad. Sci. USA *109*, 10001–10005.

Cypess, A.M., White, A.P., Vernochet, C., Schulz, T.J., Xue, R., Sass, C.A., Huang, T.L., Roberts-Toler, C., Weiner, L.S., Sze, C., et al. (2013). Anatomical localization, gene expression profiling and functional characterization of adult human neck brown fat. Nat. Med. *19*, 635–639.

de Souza, C.J., Hirshman, M.F., and Horton, E.S. (1997). CL-316,243, a beta3-specific adrenoceptor agonist, enhances insulin-stimulated glucose disposal in nonobese rats. Diabetes *46*, 1257–1263.

FDA (2012a) Advisory Committee Briefing Document Mirabegron (YM178) For the Treatment of Overactive Bladder, April 5, 2012. Available at http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/ReproductiveHealthDrugsAdvisoryCommittee/UCM298285.pdf accessed 6 November 2014).

FDA (2012b) Summary of safety and efficacy as basis for Advisory Committee briefing document for mirabegron, April 5th 2012. Division of Reproductive and Urologic Products, Office of New Drugs Center for Drug Evaluation and Research of Food and Drug Administration. Available at http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/ReproductiveHealthDrugsAdvisoryCommittee/UCM298284.pdf (accessed 6 November 2014).

Hall, K.D., Sacks, G., Chandramohan, D., Chow, C.C., Wang, Y.C., Gortmaker, S.L., and Swinburn, B.A. (2011). Quantification of the effect of energy imbalance on bodyweight. Lancet *378*, 826–837.

Hondares, E., Iglesias, R., Giralt, A., Gonzalez, F.J., Giralt, M., Mampel, T., and Villarroya, F. (2011). Thermogenic activation induces FGF21 expression and release in brown adipose tissue. J. Biol. Chem. *286*, 12983–12990.

Jespersen, N.Z., Larsen, T.J., Peijs, L., Daugaard, S., Homøe, P., Loft, A., de Jong, J., Mathur, N., Cannon, B., Nedergaard, J., et al. (2013). A classical brown adipose tissue mRNA signature partly overlaps with brite in the supraclavicular region of adult humans. Cell Metab. *17*, 798–805.

Kim, H., Pennisi, P.A., Gavrilova, O., Pack, S., Jou, W., Setser-Portas, J., East-Palmer, J., Tang, Y., Manganiello, V.C., and Leroith, D. (2006). Effect of adipocyte beta3-adrenergic receptor activation on the type 2 diabetic MKR mice. Am. J. Physiol. Endocrinol. Metab. *290*, E1227–E1236.

Larsen, T.M., Toubro, S., van Baak, M.A., Gottesdiener, K.M., Larson, P., Saris, W.H., and Astrup, A. (2002). Effect of a 28-d treatment with L-796568, a novel beta(3)-adrenergic receptor agonist, on energy expenditure and body composition in obese men. Am. J. Clin. Nutr. *76*, 780–788.

Lidell, M.E., Betz, M.J., Dahlqvist Leinhard, O., Heglind, M., Elander, L., Slawik, M., Mussack, T., Nilsson, D., Romu, T., Nuutila, P., et al. (2013). Evidence for two types of brown adipose tissue in humans. Nat. Med. *19*, 631–634.

Malik, M., van Gelderen, E.M., Lee, J.H., Kowalski, D.L., Yen, M., Goldwater, R., Mujais, S.K., Schaddelee, M.P., de Koning, P., Kaibara, A., et al. (2012). Proarrhythmic safety of repeat doses of mirabegron in healthy subjects: a randomized, double-blind, placebo-, and active-controlled thorough QT study. Clin. Pharmacol. Ther. *92*, 696–706.

Masutani, S., Cheng, H.J., Morimoto, A., Hasegawa, H., Han, Q.H., Little, W.C., and Cheng, C.P. (2013). β3-Adrenergic receptor antagonist improves exercise performance in pacing-induced heart failure. Am. J. Physiol. Heart Circ. Physiol. *305*, H923–H930.

Muzik, O., Mangner, T.J., Leonard, W.R., Kumar, A., Janisse, J., and Granneman, J.G. (2013). 15O PET measurement of blood flow and oxygen consumption in cold-activated human brown fat. J. Nucl. Med. *54*, 523–531.

Ouellet, V., Labbé, S.M., Blondin, D.P., Phoenix, S., Guérin, B., Haman, F., Turcotte, E.E., Richard, D., and Carpentier, A.C. (2012). Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. J. Clin. Invest. *122*, 545–552.

Redman, L.M., de Jonge, L., Fang, X., Gamlin, B., Recker, D., Greenway, F.L., Smith, S.R., and Ravussin, E. (2007). Lack of an effect of a novel beta3-adrenoceptor agonist, TAK-677, on energy metabolism in obese individuals: a double-blind, placebo-controlled randomized study. J. Clin. Endocrinol. Metab. *92*, 527–531.

Sacco, E., and Bientinesi, R. (2012). Mirabegron: a review of recent data and its prospects in the management of overactive bladder. Ther. Adv. Urol. *4*, 315–324.

Sacks, H.S., Fain, J.N., Bahouth, S.W., Ojha, S., Frontini, A., Budge, H., Cinti, S., and Symonds, M.E. (2013). Adult epicardial fat exhibits beige features. J. Clin. Endocrinol. Metab. *98*, E1448–E1455.

Saito, M., Okamatsu-Ogura, Y., Matsushita, M., Watanabe, K., Yoneshiro, T., Nio-Kobayashi, J., Iwanaga, T., Miyagawa, M., Kameya, T., Nakada, K., et al. (2009). High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. Diabetes *58*, 1526–1531.

Sharp, L.Z., Shinoda, K., Ohno, H., Scheel, D.W., Tomoda, E., Ruiz, L., Hu, H., Wang, L., Pavlova, Z., Gilsanz, V., and Kajimura, S. (2012). Human BAT possesses molecular signatures that resemble beige/brite cells. PLoS ONE *7*, e49452.

Stanford, K.I., Middelbeek, R.J., Townsend, K.L., An, D., Nygaard, E.B., Hitchcox, K.M., Markan, K.R., Nakano, K., Hirshman, M.F., Tseng, Y.H., and Goodyear, L.J. (2013). Brown adipose tissue regulates glucose homeostasis and insulin sensitivity. J. Clin. Invest. *123*, 215–223.

Takasu, T., Ukai, M., Sato, S., Matsui, T., Nagase, I., Maruyama, T., Sasamata, M., Miyata, K., Uchida, H., and Yamaguchi, O. (2007). Effect of (R)-2-(2-aminothiazol-4-yl)-4′-2-[(2-hydroxy-2-phenylethyl)amino]ethyl acetanilide (YM178), a novel selective beta3-adrenoceptor agonist, on bladder function. J. Pharmacol. Exp. Ther. *321*, 642–647.

Ursino, M.G., Vasina, V., Raschi, E., Crema, F., and De Ponti, F. (2009). The beta3-adrenoceptor as a therapeutic target: current perspectives. Pharmacol. Res. *59*, 221–234.

van der Lans, A.A., Hoeks, J., Brans, B., Vijgen, G.H., Visser, M.G., Vosselman, M.J., Hansen, J., Jörgensen, J.A., Wu, J., Mottaghy, F.M., et al. (2013). Cold acclimation recruits human brown fat and increases nonshivering thermogenesis. J. Clin. Invest. *123*, 3395–3403.

van Marken Lichtenbelt, W.D., Vanhommerig, J.W., Smulders, N.M., Drossaerts, J.M., Kemerink, G.J., Bouvy, N.D., Schrauwen, P., and Teule, G.J. (2009). Cold-activated brown adipose tissue in healthy men. N. Engl. J. Med. *360*, 1500–1508.

Vansal, S.S., and Feller, D.R. (1999). Direct effects of ephedrine isomers on human beta-adrenergic receptor subtypes. Biochem. Pharmacol. *58*, 807–810.

Villarroya, J., Cereijo, R., and Villarroya, F. (2013). An endocrine role for brown adipose tissue? Am. J. Physiol. Endocrinol. Metab. *305*, E567–E572.

Virtanen, K.A., Lidell, M.E., Orava, J., Heglind, M., Westergren, R., Niemi, T., Taittonen, M., Laine, J., Savisto, N.J., Enerbäck, S., and Nuutila, P. (2009). Functional brown adipose tissue in healthy adults. N. Engl. J. Med. *360*, 1518–1525.

Vosselman, M.J., van der Lans, A.A., Brans, B., Wierts, R., van Baak, M.A., Schrauwen, P., and van Marken Lichtenbelt, W.D. (2012). Systemic β-adrenergic stimulation of thermogenesis is not accompanied by brown adipose tissue activity in humans. Diabetes *61*, 3106–3113.

Weyer, C., Tataranni, P.A., Snitker, S., Danforth, E., Jr., and Ravussin, E. (1998). Increase in insulin action and fat oxidation after treatment with CL 316,243, a highly selective beta3-adrenoceptor agonist in humans. Diabetes *47*, 1555–1561.

Wu, J., Boström, P., Sparks, L.M., Ye, L., Choi, J.H., Giang, A.H., Khandekar, M., Virtanen, K.A., Nuutila, P., Schaart, G., et al. (2012). Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell *150*, 366–376.

Yoneshiro, T., Aita, S., Matsushita, M., Kayahara, T., Kameya, T., Kawai, Y., Iwanaga, T., and Saito, M. (2013). Recruited brown adipose tissue as an antiobesity agent in humans. J. Clin. Invest. *123*, 3404–3408.

Zingaretti, M.C., Crosta, F., Vitali, A., Guerrieri, M., Frontini, A., Cannon, B., Nedergaard, J., and Cinti, S. (2009). The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. FASEB J. *23*, 3113–3120.