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

Alana E. O'Mara, … , Kong Y. Chen, Aaron M. Cypess

*J Clin Invest.* 2020. [https://doi.org/10.1172/JCI131126](https://doi.org/10.1172/JCI131126).

Graphical abstract

Find the latest version:

[https://jci.me/131126/pdf](https://jci.me/131126/pdf)
Chronic mirabegron treatment increases human brown fat, HDL cholesterol, and insulin sensitivity


1Diabetes, Endocrinology, and Obesity Branch, Intramural Research Program, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA

2Liver Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA

3Diabetes and Obesity Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA, 90048

4Clinical Mass Spectrometry Core, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA

5Positron Emission Tomography Department, National Institutes of Health, Bethesda, MD 20892, USA.

6Clinical Laboratory Core, National Institute of Diabetes and Digestive Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA
7Departments of Medicine and Pediatrics, NYU School of Medicine, New York, NY 10016

8Shared contribution

Conflict of interest statement

The authors have declared that no conflict of interest exists,

Brief Summary: Treatment of healthy women with the β3-adrenergic receptor agonist mirabegron for four weeks increased brown fat metabolic activity, HDL-cholesterol, tissue glucose uptake, and insulin sensitivity.
ABSTRACT

Background. Mirabegron is a β3-adrenergic receptor (β3-AR) agonist approved only for the treatment of overactive bladder. Encouraging preclinical results suggest that β3-AR agonists could also improve obesity-related metabolic disease by increasing brown adipose tissue (BAT) thermogenesis, white adipose tissue (WAT) lipolysis, and insulin sensitivity.

Methods. We treated 14 healthy women of diverse ethnicity, 27.5 ± 1.1 y, BMI 25.4 ± 1.2 kg/m², with 100 mg mirabegron (Myrbetriq® extended-release tablet, Astellas Pharma) for four weeks, open-label. The primary endpoint was the change in BAT metabolic activity as measured by [18F]-2-fluoro-D-2-deoxy-D-glucose (18F-FDG) positron emission tomography/computed tomography (PET/CT). Secondary endpoints included resting energy expenditure (REE), plasma metabolites, and glucose and insulin metabolism as assessed by frequently sampled intravenous glucose tolerance test.

Results. Chronic mirabegron therapy increased BAT metabolic activity. Whole-body REE was higher, without changes in body weight or composition. Additionally, there were elevations in plasma levels of the beneficial lipoprotein biomarkers high-density lipoprotein (HDL) and ApoA1, as well as total bile acids. Adiponectin, a WAT-derived hormone that has anti-diabetic and anti-inflammatory capabilities, increased with acute treatment and was 35% higher at study completion. Finally, an intravenous glucose tolerance test demonstrated higher insulin sensitivity, glucose effectiveness, and insulin secretion.

Conclusion. These findings indicate that human BAT metabolic activity can be increased after chronic pharmacological stimulation with mirabegron and support the investigation of β3-AR agonists as a treatment for metabolic disease.

Trial Registration: Clinicaltrials.gov NCT03049462
Funding. This work was supported by the Intramural Research Program of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), DK075112, DK075116, DK071013, and DK071014.
INTRODUCTION

The rising rates of obesity are a global health epidemic. In the US alone, by 2030 it is predicted that 86% of adults will be overweight or obese (1). Comorbidities include impaired glucose tolerance, dyslipidemia, hypertension, and a proinflammatory state (2). Addressing these problems with behavioral interventions alone has been largely unsuccessful, highlighting the need in many patients for adjunct therapy to maintain long-term improvements in obesity-related metabolic disease (3). One complementary approach has been pharmacological treatment to increase fatty acid and glucose oxidation. For several decades, an attractive target has been the β3-adrenergic receptor (β3-AR), whose activation in rodents leads to increased energy expenditure and improved glucose tolerance (4). The mechanism has thought to include a combination of increased brown adipose tissue (BAT) thermogenesis and white adipose tissue (WAT) capacity for glucose uptake (4, 5).

Translation of the β3-AR rodent studies to humans has not been straightforward. Species differences in drug selectivity, oral bioavailability, and gene expression were limiting factors for achieving weight loss (6, 7). Nevertheless, early-phase clinical trials showed improved glucose tolerance (8) and increased fatty acid oxidation (9). Using the β3-AR agonist mirabegron (Myrbetriq®, extended-release tablet, Astellas Pharma), approved for the treatment of overactive bladder, we recently showed that a single 200 mg dose stimulated BAT glucose uptake, WAT lipolysis, and altered bile acid metabolism in healthy lean men (7). Neither the acute nor the chronic effects of mirabegron on adult women have been examined, so the current study addressed the hypothesis that four weeks of treatment with mirabegron could increase BAT metabolic activity.
RESULTS

Fourteen healthy, young women (27.5 ± 1.1 y) of mixed ethnicity (5 Black or African-American, 8 White, 1 American Indian/Alaska Native; 1 Hispanic/13 non-Hispanic) initiated the study (Figure 1A and Table 1). Each was treated for four weeks with daily oral doses of 100 mg mirabegron (Figure 1B). This dosage is higher than the maximum approved dosage (50 mg daily) and was chosen because it was more likely to stimulate BAT (7). By Day 14 of treatment, we presumed that the subjects’ β3-AR’s were being stimulated continuously, as trough plasma concentrations of mirabegron were nearly at the Ki of 55 nM (10): 40.3 ± 5.0 and 45.2 ± 4.5 nM at Days 14 and 28, respectively (Figure 2 and Supplemental Table 1). At the completion of the trial (Day 28), there were no changes in weight, fat mass, fat-free mass, or reported food intake (Table 1).

The prespecified primary endpoint, the subjects’ detectable BAT metabolic activity as measured via [18F]-2-fluoro-D-2-deoxy-D-glucose (18F-FDG) positron emission tomography/computed tomography (PET/CT), significantly increased (Figure 3, A and B and Supplemental Figure 1), median 195 to 473 mL/g/mL (P = 0.039). Similar proportional increases were seen with BAT volume (Figure 3C), median 72 to 149 mL (P = 0.036), and maximum metabolic activity (Figure 2D), median 10 to 29 g/mL (P = 0.017). The extent of changes in BAT activity and volume were not the same across the group. The women who had less BAT on Day 1 had larger increases than those who started with more (R²=0.65 and 0.71, respectively, for activity and volume, both P < 0.001)(Supplemental Figure 3, A and B). These patterns suggest that chronic mirabegron treatment is particularly effective at increasing BAT activity in subjects who had little BAT before treatment, but there may also be an upper threshold in its efficacy. Of note, while daily outdoor temperatures have been found to affect the
activity of BAT in response to cold stimulation (11), no relationship was found between the change in log_{10} BAT activity vs change in mean outdoor temperature by linear regression (R^2=0.11, P = 0.35).

The PET/CT imaging also allowed us to measure metabolic activities of other tissues that can contribute to thermogenesis. In contrast to BAT, ^18F-FDG uptake in erector spinae skeletal muscle was unchanged (-0.01 ± 0.05 g/mL, P = 0.77) and was lower in the dorsal-lumbar depot of subcutaneous WAT (scWAT) (-0.15 ± 0.04 g/mL, P = 0.006) (Figure 3, E and F). The reason for assessing scWAT glucose uptake in particular was to determine if there had been a detectable increase in thermogenic adipocytes in this very large depot. Since we did not detect any increase in muscle or scWAT activity, it suggests that the major targets of chronic exposure to a β3-AR are the adipose depots previously identified (12) (Supplemental Figure 3).

Human thermogenic adipocytes can be from two distinct lineages: constitutive “brown” in the cervical and supraclavicular regions and recruitable “beige/brite” adipocytes in the supraclavicular and abdominal depots, as well as other, smaller sites (13-18). Without biopsies, the current study could not directly distinguish between these two cell types or whether the increased metabolic activity was due to hypertrophy or hyperplasia. Based on the wide distribution of activation, it is likely that both brown and beige/brite adipocytes contributed to the higher metabolic activity (19). The increase was not uniformly distributed, with the mediastinal, paraspinal, and abdominal depots showing the largest gains (Supplemental Figure 4) (7).

To evaluate how mirabegron impacted whole-body metabolism, we used a repeated measures ANOVA to determine the effects of day of study, time, and their interaction, on resting energy expenditure (REE)(Figure 4A and Supplemental Table 2). There was a significant effect of both
time of day ($P < 0.001$) and the interaction between day of study and time of day ($P = 0.001$): the initial dose of mirabegron on Day 1 increased REE by $10.7\% \ (+6.4 \pm 1.2 \text{ kcal/h}, \ P < 0.001$), yet the Day 28 dose of mirabegron did not further increase REE above the Day 28 pre-dose baseline ($0.8\% = 0.5 \pm 1.2 \text{ kcal/h}, \ P = 0.70$). However, the baseline REE (at 08:00) on Day 28 was $5.8\%$ higher than baseline REE prior to drug exposure on Day 1 ($+82 \text{ kcal/d}, \ P = 0.01$).

The respiratory quotient (RQ) reflects substrate source. The acute dose of mirabegron on both Day 1 and Day 28, lowered the RQ ($-0.069 \pm 0.007$ and $-0.051 \pm 0.007$, respectively; both $P < 0.001$) (Figure 4B and Supplemental Table 3), indicating a net increase in fat oxidation. In contrast to the results with REE, the baseline RQ on Day 28 was not different from Day 1 ($P = 0.15$), and the $P$ value for the interaction between day of study and time of day was higher ($P = 0.06$). These changes in REE and RQ were likely due to both prolonged fasting and mirabegron itself, but the lack of a placebo group precludes us from determining the contributions from each. We also measured the energy expenditure and RQ for the entire chamber stay, from evening until the next afternoon, and saw no difference in either parameter: $74.4 \pm 8.4 \text{ kcal/h}$ to $75.0 \pm 8.0 \text{ kcal/h}$ ($P = 0.57$) and $0.850 \pm 0.023$ to $0.854 \pm 0.025$ ($P = 0.51$), respectively. There were also no changes in total sleep time or sleep efficiency: $391.8 \pm 76.6$ minutes to $354.5 \pm 65.3$ minutes ($P = 0.11$), and $89.8 \pm 8.0\%$ to $91.5 \pm 4.6\%$ ($P = 0.27$), respectively. In addition, average core temperature was $0.11 \pm 0.02 \degree \text{C}$ higher on Day 28 (Supplemental Figure 5).

A concern related to chronic treatment with adrenergic agonists is pathologic overstimulation of the cardiovascular system. Here, resting heart rate (HR), systolic blood pressure (SBP), and rate-pressure product (RPP), a correlate of myocardial oxygen consumption (20), all increased acutely on Day 1 in response to mirabegron, and baselines were higher on Day 28 (Supplemental Table 4). Given that there is no published evidence yet to support direct binding of mirabegron
to either the β1-AR or β2-AR, these findings lend further support to the mechanism whereby
mirabegron is taken up by sympathetic nerve terminals and then causes the release of
norepinephrine to bind to cardiac β1-AR’s (21). Such cardiovascular stimulation is similar to
what we previously reported at 200 mg in men (7, 22) and demonstrates why dosages higher than
50 mg are not used clinically for overactive bladder. There was a diurnal variation in the change
in HR such that mirabegron increased HR more overnight than when the subjects were awake
and moving: +6.2 ± 1.7 bpm between 19:00-22:00 vs +8.4 ± 1.5 bpm between 00:00-03:00 (P <
0.001). None of the changes in HR or RPP correlated with the changes in REE (both P > 0.05,
not shown). Per self-administered questionnaires, 1-2 subjects reported occasionally
experiencing some of the anticipated mild treatment-emergent adverse effects (23): palpitations,
headaches, bowel habit changes, and tachycardia during exercise; none of these effects were
higher than Grade 1. The dose of mirabegron on Day 28 did not increase these cardiovascular
parameters any further. The changes were not long-lasting: two weeks after stopping treatment,
plasma mirabegron was 5.4 ± 0.1 nM, and heart rates returned to values at screening
(Supplemental Figure 6). Mirabegron treatment had no effect on exercise tolerance, with no
change in the maximal oxygen uptake (VO_{2\text{max}}), maximum heart rate, or maximum wattage
achieved (Supplemental Figure 7, A-C). Chronic mirabegron treatment also did not lead to
changes in measures of liver stiffness, liver steatosis, or gallbladder volume (Supplemental
Figure 7, D-F).

The effects of acute and chronic mirabegron treatment on plasma metabolites and hormones
could be separated into three distinct patterns (Supplemental Table 5). Most metabolites
demonstrated (A) no effect or changes resulting from fasting, independent of mirabegron.
However, one subset showed (B) acute increases on Day 28 that were blunted compared to Day
non-esterified fatty acids (NEFA) and β-hydroxybutyrate. Plasma level increases of these metabolites was likely due to β3-AR stimulation of WAT. It remains to be determined whether chronic mirabegron treatment led to reduced responsiveness by the WAT or if there was increased consumption of the metabolites.

The final pattern (C) of metabolite changes in response to chronic mirabegron treatment has the greatest potential to improve metabolic health. There were increased fasting levels of the following: high-density lipoprotein (HDL) (+8%), ApoA1 (+12%), ApoE (+7%), total bile acids (+49%), total GIP (+31%), and adiponectin (+35%). In addition, there was a reduction in the ApoB100/ApoA1 ratio, a biomarker of cardiovascular risk (24). We found higher levels of both total chenodeoxycholic acid (CDCA) (1003 ± 254 to 1641 ± 359 nM, P = 0.02) and the unconjugated form (212 ± 64 to 542 ± 159 nM, P = 0.02), the latter of which may be able to further boost BAT thermogenesis (25).

Without altering body weight, the initial clinical trials using non-FDA approved β3-AR agonists provided some evidence for improved glucose tolerance (8) and insulin sensitivity (9). In addition, rodent studies showed that β3-AR-mediated activation of WAT can increase pancreatic β-cell insulin secretion (26-28). To assess both glucose metabolism and β-cell function in our study using mirabegron, we performed frequently-sampled intravenous glucose tolerance tests (FSIGT, Figure 5, A and B) and quantified metabolic parameters using Bergman’s Minimal Model (Supplemental Table 6) (29). Modeling showed that the parameters associated with insulin sensitivity, insulin-independent glucose metabolism, and insulin secretion all increased substantially (Figure 5, C-F). Whole body insulin sensitivity ($S_I$), reflecting insulin’s action to both stimulate glucose uptake and suppress endogenous glucose production, increased by 36% ($P = 0.026$). While $S_I$ includes insulin’s actions to both stimulate glucose uptake and
suppress endogenous glucose production, the effects on tissue glucose uptake are dominant (30).

Glucose effectiveness ($S_G$), the ability of glucose to mediate its own net disappearance independent of elevated insulin response, increased by 34% ($P = 0.002$).

Regarding $\beta$-cell function, the acute insulin response to glucose ($\text{AIR}_G$) increased by 37% ($P = 0.039$). The Disposition Index (DI), an overall measure of the ability of $\beta$-cells to secrete insulin when normalized to the degree of insulin resistance, increased by 82% ($P = 0.005$).

Despite the increases in both $S_I$ and $\text{AIR}_G$, there was no relationship between the changes in these two measures for each of the subjects ($P = 0.39$). The change in HOMA-IR, a measure of insulin resistance (31) was not significant after chronic mirabegron treatment ($0.09 \pm 0.81$, $P = 0.72$).
DISCUSSION

The labyrinthine paths toward developing pharmacological activators of β3-AR’s and BAT thermogenesis to treat human metabolic disease have much in common. Both have been bolstered by decades of highly encouraging preclinical studies (4, 5, 8, 9), yet to date, neither has achieved its goal. A major step forward for the β3-AR was the recent approval of mirabegron, the first highly selective agonist, to treat overactive bladder (23). A single dose of mirabegron stimulates BAT glucose uptake (22), a marker of thermogenesis, along with increases in WAT lipolysis, REE, and changes in plasma bile acids (7). In this study, we report the physiological effects of chronic daily oral treatment with mirabegron. The numerous potentially beneficial metabolic responses now require determining which are attributable directly to mirabegron and which are downstream of the initial effects.

Chronic mirabegron treatment increased BAT metabolic activity, the primary endpoint, and this approach now joins chronic cold exposure as another way to augment BAT thermogenesis (32-34). Two distinct facets of our findings relate to where and in whom BAT increased. The preferential amplification of the perirenal depots is particularly noteworthy because of the recent identification there of unilocular, dormant brown adipocytes (35). These cells express the β3-AR at much higher levels than scWAT and respond to treatment with adrenergic agonists by increasing their thermogenic capacity. In addition, these dormant cells have less sympathetic innervation, which could make them comparatively more responsive to pharmacological activation through the blood than through cold exposure, which relies on sympathetic neurons. Additional studies are needed to validate this model and determine if it applies to the mediastinal and paraspinal depots as well.
The favorable changes in multiple lipoproteins and bile acids may also be connected to BAT activation. A potential mechanism comes from a series of recent studies in mice showing that when BAT is stimulated chronically, it consumes triglyceride-rich lipoproteins and subsequently generates HDL cholesterol. HDL is then taken up and converted in the liver to bile acids, particularly via the alternative pathway that preferentially synthesizes CDCA (36-39). The plasma bile acids then bind to farnesoid X receptor (FXR) and TGR5 receptors and exert a combination of metabolically beneficial effects through the liver, intestine, microbiome, and mononuclear phagocytic system, including the production of incretins such as GIP (40, 41).

Future studies are required to corroborate the mechanisms by which the observed changes in lipoproteins, adipokines, and bile acids are achieved and to conclusively demonstrate if these effects represent new, physiologically relevant roles for BAT in human metabolism.

Besides effects on BAT, HDL cholesterol, and bile acids, mirabegron also produced substantial improvements in glucose and insulin metabolism. In context, the increases we observed in $S_G$, $S_I$, $AIRG$, and $DI$, between 30-90%, were generally in the range of interventions associated with improvements in insulin sensitivity or pancreatic $\beta$-cell function: an acute dose of iv GLP-1 ($S_I +4\text{-}29\%$, $AIRG +48\text{-}66\%$) (42, 43); chronic metformin treatment in women with polycystic ovary syndrome ($S_G +30\%$, $S_I +2.5\%$, $AIRG +43\%$, $DI +72\%$) (44); 9-15 months after bariatric surgery ($S_I +116\%$, $AIRG -56\%$) (45); and six weeks of mild exercise training in healthy adults ($S_G +28\%$, $S_I +32\%$) (46). The improvements in glucose metabolism with mirabegron treatment were not associated with changes in either fasting glucose or insulin. Similarly, iv GLP-1 (42, 43) and exercise (46) did not raise or lower either fasting glucose or insulin levels, while metformin lowered fasting glucose (44). Therefore, preliminary evidence suggests that
mirabegron’s effects on glucose metabolism, HDL cholesterol, and bile acids resemble those achieved through mild exercise (47, 48).

As mentioned above, the changes in SI and AIRG were not correlated. This discordance may have been because mirabegron likely affects insulin sensitivity and β-cell function via different mechanisms that are not directly connected. One reason for significant improvements in SI and Sg, but not the HOMA-IR, may have been that the subjects here had near-normal HOMA-IR at study initiation (1.75 ± 0.69), and HOMA-IR does not provide a precise estimate of peripheral insulin action in this population (49). Any significant effects of mirabegron on glucose metabolism would therefore likely be detected only when glucose homeostasis was strongly perturbed, such as in the setting of the FSIGT (50, 51).

How mirabegron improved glucose metabolism is not known. Contributions could have been from the higher plasma adiponectin (52), a WAT-derived adipokine that is associated with higher insulin sensitivity in skeletal muscle (53) and liver (54). The elevated levels of the incretin GIP can also improve glucose-stimulated insulin secretion (55). A third, parallel mechanism may involve the β-cells themselves. While we did not detect β3-AR mRNA in human islets (data not shown), there is evidence that WAT lipolysis (26, 27, 56) followed by activation of β-cell fatty acid receptor GPR40, can stimulate insulin release (57). In summary, beneficial metabolic changes caused by chronic mirabegron treatment may come from stimulation of the β3-AR on human BAT and WAT.

These findings must be considered in context of the principal limitations of this study: it comprised a small group of young, healthy women with a narrow BMI range of diverse ethnicity, and each was treated with active drug and served as her own control. Future studies are needed using placebo controls in order to better determine whether the changes in BAT and other
physiological markers are dependent on mirabegron and what the contribution of mirabegron
was to the changes seen vs time and day. In addition, studies are needed in other populations,
such as the elderly and patients with metabolic disease. Also, while the FSIGT is a useful tool
for initial inquiries into insulin sensitivity and β-cell function, further studies are required to
identify tissue sites of improved metabolism. Finally, mirabegron’s beneficial effects were seen
at a dosage higher than approved by the FDA, so the outcomes here cannot yet be applied to
patients. In particular, the elevations in HR, SBP, and myocardial oxygen consumption with this
dosage of mirabegron may confer too great a cardiovascular risk for clinical treatment of
metabolic dysfunction in obese patients. However, there are other β3-AR agonists in late-stage
clinical trials that may have greater β3/β1-AR selectivity (58, 59). Such drugs may improve
metabolism with an acceptable side effect profile.

In conclusion, we demonstrated that chronic treatment for four weeks with the β3-AR agonist
mirabegron in healthy, young women with a range of BMI’s led to increases in BAT metabolic
activity; REE; plasma HDL and associated lipoproteins; insulin sensitivity; and pancreatic β-cell
insulin secretion. These metabolic benefits occurred without changes in the subjects’ weight, fat
mass, or lean body mass. Therefore, chronic activation of the β3-AR may be an effective way to
treat metabolic disease.
METHODS

Sample size calculation

Our previous studies showed that BAT activity was not normally distributed; rather, it is right skewed (7, 22). Thus, our sample size determination was based on log$_{10}$BAT activity. Since there were no published studies assessing the effects of mirabegron on log$_{10}$BAT activity, we used a similar cohort exposed to chronic cold exposure (32) that reported changes in log$_{10}$ BAT volume of $+0.21 \pm 0.25$ mL. Since BAT volume correlates with BAT activity (12), we then determined that a sample size of 14 female subjects was necessary to detect whether a change in BAT metabolic activity was different from a null hypothesis of 0.00 with 80% power using a paired Student’s $t$-test with a significance level of 0.05 and a correlation (R) of 0.50.

Treatment was nonblinded for both staff and subjects.

Subject Information and Protocol Design

Inclusion criteria were the following: generally healthy women between the age 18-40 years. Exclusion included were as described (https://clinicaltrials.gov/ct2/show/NCT03049462). Only women were recruited because they were not included in our previous studies, which used a 200 mg mirabegron dose that can cause QT prolongation, values above 450 ms, in women but not men (60). The protocol included a screening visit at which time we conducted a medical history and physical examination, measured metabolites and hormones, and assessed the heart rhythm via electrocardiogram (ECG); two study visits (Day 0-1 and Day 27-28); and a follow-up safety visit to re-assess heart rate and rhythm, inquire about any adverse effects, and measure plasma mirabegron, as described below and Figure 1A. In practice, “Day 28” was $28 \pm 0.4$ d after Day 0, and the follow-up safety visit was $16 \pm 0.7$ d after Day 28. Mean daily temperatures in
Bethesda, MD, for the 20 months of the study were obtained from the National Oceanic and Atmospheric Administration (https://www.ncdc.noaa.gov/cdo-web/search) and are displayed in Supplemental Figure 8 in relation to the subjects’ individual study days. When a specific day’s temperature was not available, we used the day that was closest in time to the actual study day.

Twenty-two subjects enrolled; one was ineligible to participate; and seven either withdrew or were lost to follow-up prior to initiation. All 14 subjects who initiated the study completed it. Four of the 14 subjects reported travel outside of the Bethesda, MD, area during the study period and could not be included in subsequent analyses related to outdoor temperature. Subjects were asked to keep consistent weight-maintenance diets and exercise habits over the course of the study. All 14 subjects who initiated the study completed it. There was high daily adherence to medication administration, which was monitored using the Nomi Real-time medication adherence technology (SMRxT) and also assessed by measuring trough concentrations of mirabegron at two weeks and four weeks after study Day 1.

Metabolic Testing: Day 0 & Day 27

Healthy volunteers who passed screening were admitted to the Metabolic Clinical Research Unit (MCRU) at the NIH Hatfield Clinical Research Center the evening before testing at the initiation (Day 0) and completion (Day 27) of the study. The goal was to study all subjects on Days 0/1 and Days 27/28 on the same day of their menstrual cycles. In practice, the difference in days of the cycle between Days 0/1 and Day 27/28 was -0.6 ± 2.3 d. Of the 14 subjects, 12 were studied within the first three weeks of their menstrual cycles. During the evenings prior to the FSIGT and the BAT imaging, subjects ate a weight-maintenance, caffeine-free dinner of 716 ± 111 kcal with a macronutrient distribution of 55% carbohydrate, 15% protein, and 30% fat (61,
Prior to 12:00 am, they were provided a high-carbohydrate, high-protein snack that was 251 ± 49 kcal. Inpatient rooms were kept at 24 °C, and subjects were asked to go to sleep at the same time each night. Clothing was standardized during each metabolic chamber stay as described (12).

Subjects fasted from 00:00 until completing testing later that day (Day 0/Day 27). Subjects first underwent a frequently-sampled intravenous glucose tolerance test (FSIGT) from 08:00-11:00. At time 0, participants received an intravenous bolus of glucose (0.3 g/kg body weight). At 20 minutes, a bolus of insulin (0.03 U/kg body weight) was administered. Blood samples (~2 mL) for glucose and insulin were taken at –10, -1, 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 20, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 120, 150, and 180 minutes. Afterward, subjects met with a dietician to review food intake records and collect anthropometric measures (neck, waist, hip, arm, and leg circumference and length) in triplicate. Liver stiffness and steatosis were assessed using vibration controlled transient elastography (VCTE) and continuous attenuation parameter (CAP), respectively, with the Fibroscan device (Echosens, Waltham, MA) using the M probe. Ten valid measurements were obtained, and the median result was used. Afterwards, subjects ate an isocaloric lunch and underwent ECG testing. At 15:00, subjects performed a VO\(_{2\text{max}}\) test to measure volitional fatigue on a cycle ergometer under a 15-20 W/min continuous ramp protocol. At 17:00, a dual-energy X-ray absorptiometry (DXA) scan was performed (iDXA, GE Healthcare, Madison WI). At 18:00, subjects entered the metabolic chamber.

Quantification of Metabolic Activity, Physiological, and Clinical Measurements

Subjects stayed in the metabolic chamber for 20 hours (18:00-14:00) during which energy expenditure and respiratory quotient were measured via indirect calorimetry from oxygen.
consumption and carbon dioxide production and ECG was recorded by a Holter monitor (Del Mar-Reynolds, Irvine, CA, USA). Energy expenditure and RQ were calculated for the entire 20-hour chamber stay, and the first and last 30 minutes were excluded in determining the overall energy expenditure and RQ. At 18:00, they were provided an isocaloric dinner and snack; they fasted from 00:00 until exiting the chamber the following day (Day 1/Day 28). Thirteen of 14 enrolled subjects completed the full overnight stay in the metabolic chambers. One subject entered the metabolic chamber at 08:00 on Day 1/28 instead of 18:00 the prior night because of scheduling constraints. Heart rate, and blood pressure, were determined as described (7, 63). Core temperature was measured by the participant using a handheld infrared tympanic thermometer (PRO4000, Braun, Melsungen, Germany).

The relative humidity of each chamber was controlled between 30-50%. The chamber’s temperature was set to 26 °C to ensure that BAT was not activated by cold exposure. While in the chamber, subjects were asked to minimize their physical activity and stay awake other than during their nightly sleep period. Sleep actigraphy was measured using triaxial accelerometers (Actigraph GT3X+, Actigraph LLC, Pensacola, FL) worn on the wrist. We used the Cole-Kripke algorithm implemented in ActiLife software (version 6.12.0) to detect time-in-bed, sleep onset, and awakenings and recorded total minutes in bed, total sleep time, and sleep efficiency, which is the ratio of the two (64).

Between 08:25-08:55 and 13:10-13:40, volunteers sat upright and still, without any physical activity. These inactive periods provided motion-free data to calculate REE and resting heart rate. Study subjects were administered 100 mg mirabegron at 09:00 (+0 minutes). Blood samples used to measure blood metabolites were obtained 60 minutes prior to mirabegron dosing (08:00) and then 60 minutes prior to exiting the metabolic chamber (13:00). Blood samples used
to test for plasma mirabegron concentrations were obtained at +0, +30, +60, +120, +180, +210, +240, +270, +300, and +360 relative to the time of drug administration (09:00). Four hours after mirabegron administration (13:00), volunteers were injected with a 185 MBq (5 mCi) bolus of $^{[18}F]-2$-fluoro-D-2-deoxy-D-glucose (FDG) for PET/CT scanning, after which subjects spent an additional 60 minutes inside the chamber with limited physical activity to allow for uptake of the radioactive tracer.

PET/CT images were acquired and analyzed as described (12, 65). In brief, PET/CT images were reconstructed into image voxels of $1.45 \times 1.45 \times 1.5$ mm for PET and of $0.98 \times 0.98 \times 1.5$ mm for CT and uploaded into ImageJ for image processing (66). The PET/CT Viewer plug-in with features customized for BAT quantification was used in each of the subsequent analyses. Specific CT density ranges were used to identify fat (-300 to -10 HU) from air and other tissues. $^{18}$F-FDG uptake (g/mL) in each PET image voxel was quantified as an SUV initially normalized to the individual’s lean body mass. Both PET SUV and CT HU criteria were met to identify metabolically active adipose tissue.

BAT metabolic activity, defined as BAT volume multiplied by SUV mean, where SUV mean is the average radioactivity concentration in BAT divided by injected FDG dose per body weight. It, along with BAT volume; BAT, skeletal muscle, and scWAT SUV$_{max}$; and gallbladder volume were quantified as previously described (7, 12, 65): one ROI was created on each axial slice, avoiding regions that were not metabolically active fat in order to minimize false-positive detection. ROI selection began at the slice corresponding to vertebra C3 and continued inferiorly until the umbilicus. All axial ROIs were summed to calculate total body BAT volume and activity, and SUVs were averaged to determine the SUVmean. The study parameters have also
been summarized in Supplemental Tables 7-10 according to the Brown Adipose Reporting
Criteria in Imaging STudies (BARCIST 1.0) criteria (67).

Measurement of Plasma Mirabegron and Individual Bile Acid Concentrations via UPLC-MS/MS

Detection and quantification of mirabegron were achieved by ultra-performance liquid chromatography - tandem mass spectrometry (UPLC-MS/MS) utilizing a Thermo Scientific Vanquish UPLC with a Thermo Scientific Atlas triple quadrupole mass spectrometer as described previously (7). Individual bile acid detection and quantification were achieved utilizing a Thermo Scientific Vanquish UPLC with a Thermo Scientific Atlas triple quadrupole mass spectrometer, heated electrospray ionization (HESI-II) in negative ion mode (2500 V). Quantitation of bile acids and internal standard were based on the retention time and m/z.

Calibration stock solutions and internal standard $^2$H$_4$-CDCA (10 ng/nL) were prepared in MeOH and stored at 4 °C. 50 µL calibration stock standards were mixed with 50 µL H$_2$O and 150 µL internal standard for LC-MS analysis, plasma samples were prepared by protein precipitation with 50 µL plasma mixed with 50 µL MeOH and 150 µL internal standard, vortexed and then centrifuged at 4 °C, 14,000 rpm for 15 minutes. The supernatant was transferred to an LC-MS vial. Injection volume was 2 µL injection.

High-performance liquid chromatography (HPLC) grade solvents and LC-MS modifiers were purchased from Sigma-Aldrich (St. Louis, MO, USA). A Waters Acquity UPLC BEH C18, 2.1 x 100 mm, 1.7 µm column was maintained at 40 °C. Solvent A: H$_2$O, 5 mM NH$_4$OAc with 0.012% FA, and Solvent B: MeOH, 5 mM NH$_4$OAc with 0.012% FA. The flow rate was 400 µL min$^{-1}$, the gradient was started 30% B from 0 min to 0.5min, increased to 95% B at 12 min,
maintained 95% B to 14 min, then returned to 30% B at 15 min to 18 min. Bile acids standards were analyzed, and the calibration curve had a minimum $R^2 \geq 0.99$ with $1/x$ weighting.

Measurement of Metabolites, Hormones, and Adipokines

Glucose, insulin, non-esterified fatty acids (NEFA), glucagon, growth hormone, total T3, free T4, TSH, ACTH, PTH, protein, creatine kinase, pyruvate, lactate, cortisol, norepinephrine, epinephrine, and dopamine were measured by the NIH Department of Laboratory Medicine. For epinephrine and dopamine concentrations that were below the assay detection limit, we imputed the half-minimum value. Lipid profile (total cholesterol, high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C], and triglycerides [TG]), were measured using the Roche Cobas 6000 analyzer (Roche Diagnostics, Indianapolis, IN).

Metabolic proteins and hormones were measured at the NIDDK Clinical Core Lab. A colorimetric assay kit was used to measure β-hydroxybutyrate (Cayman Chemical). Total bile acids were measured in plasma using the NBT kit from Diazyme (Poway, CA). FGF-19, leptin, and adiponectin were measured using Quantikine ELISA kits (R&D Systems). Active GLP-1, active GIP, active Ghrelin, total PYY, total GIP, and FGF-21 were measured in plasma containing DPP-IV and protease inhibitors from Sigma-Aldrich (St Louis, MO) using immunoassay kits from Meso Scale Discovery (Rockville, MD). ApoA1, ApoE, ApoB100, and ApoC3 were measured in plasma samples using the Milliplex (Billerica, MA) Human Apolipoprotein Magnetic Bead Panel based on Luminex xMAP technology.

Human Islet Studies
Islets from previously non-diabetic or type 2 diabetic human cadaver donor pancreases were obtained via the NIDDK-sponsored Integrated Islet Distribution Program (Duarte, CA) as described (68). mRNA was extracted and quantified via qPCR as described (7).

Statistical methods

After completing the study in 14 subjects with detectable BAT, data were analyzed with JMP 13.0.0 software (SAS Institute, Inc.) and Graph Pad Prism 7.0 (GraphPad Software, Inc.). To evaluate the primary endpoint, we used a paired Student’s t-test on log-transformed data, which was the prespecified analysis. All other paired comparisons were performed using either paired Student’s t-tests or repeated measures ANOVA, with the assumption that the underlying distributions were normal. Simple linear regression was used to determine how the difference in mean outdoor temperature between Day 1 and Day 28 affected log_{10} BAT activity. All P values are two-tailed, with statistical significance being P values ≤ 0.05 for comparisons associated with the anthropometric, pharmacokinetic, bioenergetic, and glucometabolic measures. For the behaviors of the 38 metabolites and hormones in Supplemental Table 5, the critical P value was determined using a Benjamini-Hochberg false discovery rate Q=0.25 (69).

Study approval

This clinical trial was registered with ClinicalTrials.gov (NCT03049462) and has the FDA Investigational New Drug registration number 116246. It was approved by the Human Studies Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the NIH Radiation Safety Committee. Healthy volunteers were recruited
by word of mouth or through the Patient Recruitment and Public Liaison Office of the Clinical Center and provided written informed consent according to Declaration of Helsinki principles.
Author Contributions

AEO, JWJ, JDL, RJB, ASB, KYC, AMC were responsible for the experimental design. AEO, JWJ, HC, PJW, AMC wrote the manuscript. AEO, JWJ, LAF, YF, NK, ASB, BPL, CMM, WD, and PH quantified tissue metabolic activity. AEO, JWJ, JDL, RJB, SM, LAF, TC, KYC, and AMC performed the physiological measurements. DK and YR assessed liver fat and stiffness. HC, NBJ, and PJW measured plasma mirabegron and bile acid levels. JWJ, FP, M A, RNB, AMC performed and analyzed the frequently sampled glucose tolerance tests. CC, ZAS quantified human pancreatic β-cell mRNA expression. JWJ and AMC performed the biostatistics. All authors contributed to editing the manuscript. AEO and JWJ contributed equally to this work; AEO was primarily responsible for organizing and interpreting the clinical data and is therefore listed first.

Acknowledgements

This work was supported by the Intramural Research Program of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), DK075112, DK075116, DK071013, and DK071014. We thank the excellent support provided by the NIH Metabolic Clinical Research Unit nursing team; NIH Clinical Center Nutrition Department; NIDDK Clinical Laboratory Core Lab; NIH Department of Laboratory Medicine; research pharmacy; and PET technologists. We thank Douglas Rosing for being the independent internal data safety monitor. We offer additional gratitude to Wouter van Marken Lichtenbelt for sharing his team’s data so that we could perform our sample size calculation; Sushil Rane and his lab for the gift of the human islets; Paul Wakim and Sungyoung Auh for their biostatistical advice; Marc Reitman, Jack Yanovski, and Clifton Bogardus for their discussions about the data and their implications; Anne Sumner, Sushil Rane,
and Gordon Weir for their guidance in interpreting the FSIGT data; and David Sacks and Jeff Basilio for their advice in measuring tissue glycemia. We are especially thankful to our volunteers for their commitment to our study.

Address correspondence to: Aaron M. Cypess, Diabetes, Endocrinology, and Obesity Branch, Intramural Research Program, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA. Phone: 301-435-9267; Email: aaron.cypess@nih.gov


### Table 1. Anthropometric Values at Study Initiation and Completion

<table>
<thead>
<tr>
<th>Characteristic (Unit)</th>
<th>Day 0 Value(^A)</th>
<th>Day 27 Value(^A)</th>
<th>Paired (t)-test(^B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>163.3 ± 1.5</td>
<td>163.5 ± 1.5</td>
<td>0.36</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.5 ± 3.2</td>
<td>67.8 ± 3.3</td>
<td>0.40</td>
</tr>
<tr>
<td>Food Intake (kcal/d)</td>
<td>1938 ± 139</td>
<td>1854 ± 149</td>
<td>0.50</td>
</tr>
<tr>
<td>Body Mass Index (kg/m(^2))</td>
<td>25.4 ± 1.2</td>
<td>25.4 ± 1.2</td>
<td>0.86</td>
</tr>
<tr>
<td>Body Surface Area (m(^2))(70)</td>
<td>1.75 ± 0.04</td>
<td>1.75 ± 0.05</td>
<td>0.46</td>
</tr>
<tr>
<td>Body Fat (kg)</td>
<td>23.5 ± 2.1</td>
<td>23.6 ± 2.1</td>
<td>0.54</td>
</tr>
<tr>
<td>Percent Fat (%)</td>
<td>35.3 ± 1.6</td>
<td>35.2 ± 1.6</td>
<td>0.69</td>
</tr>
<tr>
<td>Fat-free Mass (kg)</td>
<td>43.9 ± 1.5</td>
<td>44.3 ± 1.5</td>
<td>0.24</td>
</tr>
<tr>
<td>Lean Mass (kg)</td>
<td>41.7 ± 1.4</td>
<td>41.9 ± 1.4</td>
<td>0.44</td>
</tr>
<tr>
<td>Percent Lean Mass (%)</td>
<td>62.4 ± 1.5</td>
<td>62.4 ± 1.5</td>
<td>0.72</td>
</tr>
<tr>
<td>Bone Mineral Content (kg)</td>
<td>2.40 ± 0.07</td>
<td>2.41 ± 0.07</td>
<td>0.21</td>
</tr>
</tbody>
</table>

\(^A\)Mean ± SEM
Figure 1. Flow diagram describing the numbers and disposition of study subjects.

(A) Flow diagram consisting of a frequently-sampled intravenous glucose tolerance test (FSIGT), liver scan, exercise tolerance test (VO\textsubscript{max}), dual-energy X-ray absorptiometry (DXA), then entered the metabolic chamber at 18:00 and remained overnight and through 14:00 of Day 1/Day 28, after which they were transported to the PET/CT suite. Blood was drawn to measure metabolites before treatment on Day 1/Day 28 at 08:00 and then just prior to \textsuperscript{18}F-FDG administration in the chamber at 13:00. Mirabegron was administered at 09:00. The black bars above the diagrams refer to the 30-minute still periods at which resting energy expenditure (REE) was measured. The black arrows indicate the time points at which blood was drawn for pharmacokinetic measurements.
Figure 2. Mirabegron pharmacokinetics. Plasma concentration of mirabegron during Day 1 (black circles, dashed black line), interim visit Day 14 (blue triangle), and Day 28 (green squares, green line). The $K_i$ of mirabegron is 55 nM. Values represent mean ± SEM. $n = 12$. * indicates paired $t$-tests with $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. 

FDG Injection
Figure 3. Effects of chronic mirabegron treatment on BAT, skeletal muscle, and subcutaneous WAT. (A) PET images on Day 1 and Day 28 in two representative subjects; magenta arrowheads point to the supraclavicular BAT depot with low (top) and high (bottom) initial BAT FDG-uptake after acute dose of mirabegron. Detectable BAT (B) metabolic activity and (C) volume in subjects on Day 1 (black circles) and Day 28 (black squares). Both y-axes are shown using a log$_{10}$ scale. $P$ values are for the paired Student’s $t$-test on the log$_{10}$-transformed data, the prespecified analysis. SUV$_{max}$ from PET scans taken on Day 1 and Day 28 of the subjects’ (D) BAT, (E) erector spinae skeletal muscle, and (F) dorsolumbar subcutaneous WAT. Individual volume measured on Day 1 (black circles) and Day 28 (black squares); red bars represent group medians for BAT and means for skeletal muscle and WAT. $P$ values are for the paired Student’s $t$-tests. $n = 14.$
Figure 4. Effects of chronic mirabegron treatment on REE and RQ. Changes in (A) REE and (B) RQ in response to 100 mg oral mirabegron on Day 1 (circles) and Day 28 (squares) as measured in a metabolic chamber during 20-minute still periods at 08:00 (white circles and squares) and 13:00 (black circles and squares). Red bars represent group means. Repeated measures ANOVA was used to determine the effects of day of study, time, and their interaction. \( n = 14 \).
Figure 5. Frequently sampled intravenous glucose tolerance test outcomes. Plasma (A) glucose and (B) insulin responses during a frequently sampled intravenous glucose tolerance test on Day 0 (circles, black line) and Day 27 (squares, green line); error bars indicate SEM. The inset in (a) displays the levels from 0 to 10 minutes after injection of glucose. Individual changes in (C) glucose effectiveness ($S_G$), (D) whole-body insulin sensitivity index ($S_I$), (E) acute insulin response to glucose (AIR$_G$), and (F) the Disposition Index (DI). Red bars represent group means. Comparisons for each time point in (a) and (b) and between Day 0 (white circles) and Day 27 (white squares) in (c-f) were done with via paired Student's t-test, * $P < 0.05$. $n = 12$. 