

Activation of Human Brown Adipose Tissue by a β 3-Adrenergic Receptor Agonist

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SUMMARY

Increasing energy expenditure through activation of endogenous brown adipose tissue (BAT) is a potential approach to treat obesity and diabetes. The class of β 3-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 β 3-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 β 3-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 β 3-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 β 1-AR that caused undesirable cardiovascular effects (Arch, 2011). More fundamentally, it was unknown if the β 3-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 β 3-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 β 3-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.

^aBaseline values determined prior to the dosing of mirabegron.^bValues are mean ± SEM.^cMeasured 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 (R² = 0.00, p = 0.97), systolic BP (R² = 0.01, p = 0.14), or fat-free mass (R² = 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

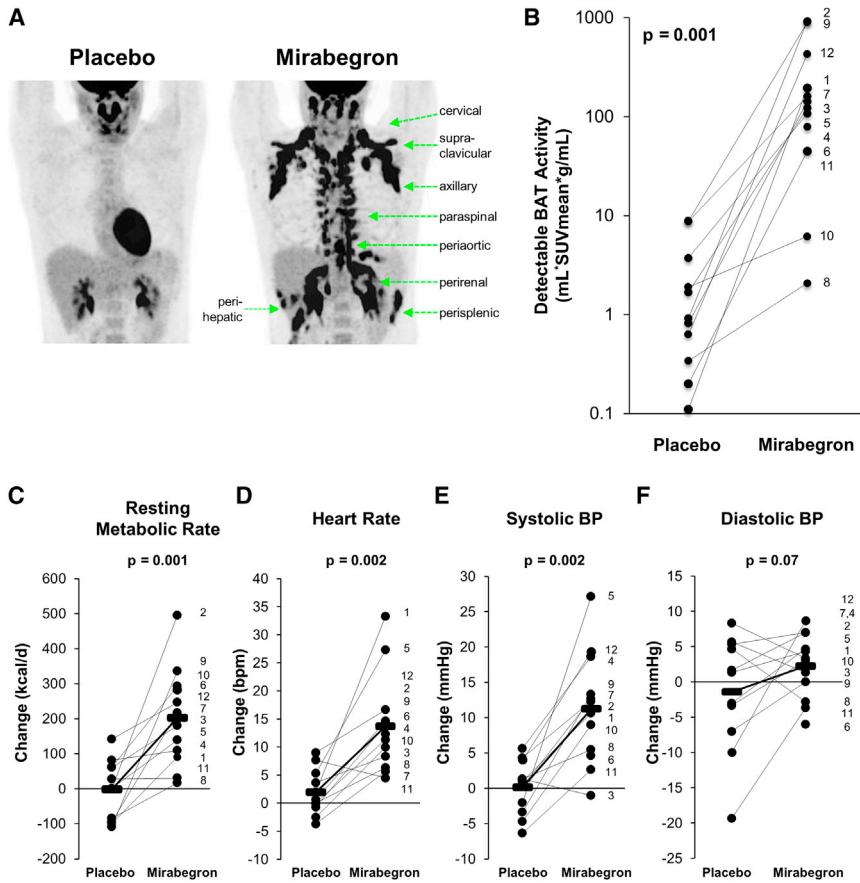


Figure 1. Metabolic Effects of the β 3-AR Agonist Mirabegron

(A) PET images of a 21-year-old man who was given placebo (left) or 200 mg of the β 3-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 β 3-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.

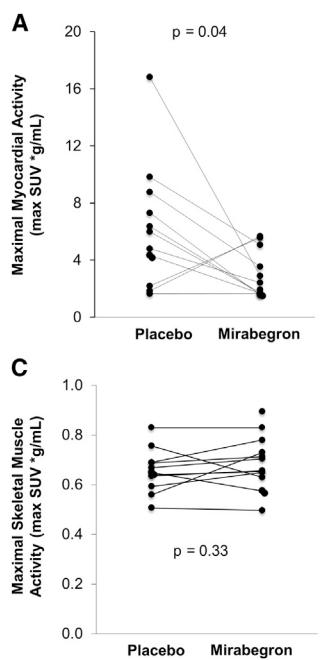
However, it is important to consider that in rodent models, treatment with β 3-AR agonists chronically can improve glucose disposal and increase RMR before a reduction in body weight is seen (de Souza et al., 1997). This distinction between the acute and chronic effects of treatment with mirabegron applies 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 β 3-AR agonists. In the present study, in addition to the effects on BAT, there was evidence for β 3-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 (Vosslman et al., 2012). Thus, chronic treatment with a β 3-AR agonist in humans may improve multiple fac-

ets 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 β 3-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 β 3-AR (Takasu et al., 2007; FDA 2012a). As a result, we observed binding to the β 1-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 β 3-AR has limited chronotropic effects (Masutani et al., 2013), concomitant administration of 200 mg mirabegron with a β 1-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 β 3-AR.

In summary, we demonstrate that the β 3-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](#).

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

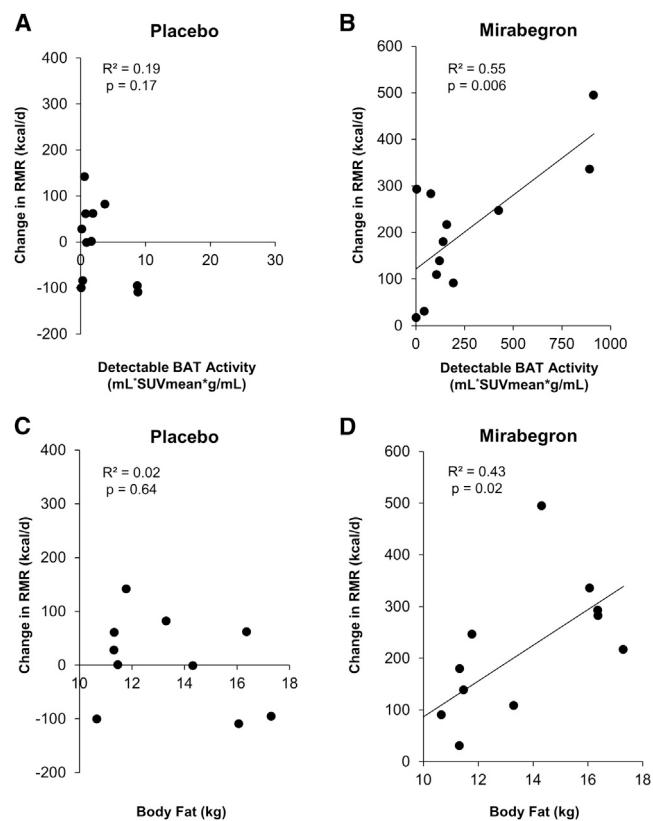
Additional information can be found in [Supplemental Experimental Procedures](#).

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

**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](#).

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 [$^{13}\text{C}_6$]-Mirabegron (Alsamim) 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 $2,361 \times g$ 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 [$^{13}\text{C}_6$] was used as 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, NEFA, 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.

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