**ORIGINAL ARTICLE**

# The effect of mirabegron on energy expenditure and brown adipose tissue in healthy lean South Asian and Europid men

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**Abstract**

**Aim:** To compare the effects of cold exposure and the β3-adrenergic receptor agonist mirabegron on plasma lipids, energy expenditure and brown adipose tissue (BAT) activity in South Asians versus Europids.

**Materials and Methods:** Ten lean Dutch South Asian (aged 18-30 years; body mass index [BMI] 18-25 kg/m²) and 10 age- and BMI-matched Europid men participated in a randomized, double-blinded, cross-over study consisting of three interventions: short-term (~ 2 hours) cold exposure, mirabegron (200 mg one dose p.o.) and placebo. Before and after each intervention, we performed lipidomic analysis in serum, assessed resting energy expenditure (REE) and skin temperature, and measured BAT fat fraction by magnetic resonance imaging.

**Results:** In both ethnicities, cold exposure increased the levels of several serum lipid species, whereas mirabegron only increased free fatty acids. Cold exposure increased lipid oxidation in both ethnicities, while mirabegron increased lipid oxidation in Europids only. Cold exposure and mirabegron enhanced supraclavicular skin

temperature in both ethnicities. Cold exposure decreased BAT fat fraction in both ethnicities. After the combination of data from both ethnicities, mirabegron decreased BAT fat fraction compared with placebo.

**Conclusions:** In South Asians and Europids, cold exposure and mirabegron induced beneficial metabolic effects. When combining both ethnicities, cold exposure and mirabegron increased REE and lipid oxidation, coinciding with a higher supraclavicular skin temperature and lower BAT fat fraction.

**KEYWORDS**
brown adipose tissue, energy expenditure, lipid metabolism, metabolic disease, mirabegron, South Asian

# 1 | INTRODUCTION

Obesity and associated diseases, including type 2 diabetes and cardiovascular diseases, are a major public health problem worldwide.[1] Certain ethnic subgroups, such as South Asian, are particularly vulnerable to developing cardiometabolic disease. This is probably, at least in part, a result of their disadvantageous metabolic profile, consisting of a susceptibility to developing abdominal obesity, dyslipidaemia and insulin resistance.[2–4] The underlying mechanisms that explain this susceptibility are not fully understood but may involve differences in skeletal muscle metabolism, size of metabolic organs and regulation of adipocytokines in South Asians.[5–8] Consequently, treatment options to improve the metabolic profile of the South Asian population are limited and unfocused, and specific strategies are needed.

Activation of brown adipose tissue (BAT) is an interesting therapeutic strategy to improve energy metabolism. BAT takes up triglyceride (TG)-derived fatty acids (FA)[9] and glucose from the systemic blood supply for combustion into heat, thereby increasing energy expenditure and improving lipid and glucose metabolism.[10,11] BAT is strongly innervated by the sympathetic nervous system. Cold exposure, resulting in sympathetic nervous system activation, is a potent physiological activator of BAT. Upon sympathetic nervous system activation, noradrenalin released from sympathetic nerve endings[12] acts on the β-adrenergic receptors (β-AR) of brown adipocytes to promote thermogenesis.[13–15] Simultaneously, BAT releases endocannabinoids, which are believed to inhibit noradrenalin signalling to prevent excessive activation of BAT.[16,17] Circulating endocannabinoid levels are elevated in obesity[18–20] and, interestingly, South Asians have higher basal circulating endocannabinoid levels compared with Europids.[21] This might, at least partly, explain the reduced $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) uptake by BAT and lower resting energy expenditure (REE) observed in South Asians compared with Europids during cold exposure.[22] In addition, the cold-induced increase in free fatty acid (FFA) levels, which generally results from lipolysis induced by sympathetic stimulation of white adipose tissue, is lower in South Asians compared with Europids.[22] Taken together, these data suggest that South Asians

have a lower sympathetic outflow upon cold exposure compared with Europids.

Repetitive cold exposure is an effective strategy to enhance BAT metabolism, as cold acclimation increases BAT volume and even reduces fat mass in healthy lean men.[23] However, as a treatment or even lifestyle involving prolonged cold exposure may be hard to adhere to, current research is focused on pharmacological compounds that can activate BAT. As BAT activation by cold is considered to occur via sympathetic stimulation of β-ARs, agonists of such receptors may be a potent way in which to activate BAT. Indeed, preclinical studies have shown that treatment with the selective β3-AR agonist CL316,243 strongly stimulates BAT activity, prevents fat accumulation, improves dyslipidaemia and insulin sensitivity, and attenuates the development of atherosclerosis.[24] Likewise, in humans, the β3-AR agonist mirabegron increased $^{18}$F-FDG uptake by BAT as well as REE in healthy young men.[25,26]

The aim of the current study was to assess the effects of cold exposure and the β3-AR agonist mirabegron on serum lipids, energy expenditure and BAT fat fraction and to compare these in healthy lean South Asian versus Europid men.

# 2 | MATERIALS AND METHODS

For more details of methods, see the supporting information.

## 2.1 | Participants

Ten healthy, young (aged 18-30 years), lean (body mass index [BMI] 18-25 kg/m$^2$) Dutch South Asian men and 10 age- and BMI-matched Europid men were included in the study. The study (clinical trial registration number: NCT03012113) was approved by the Medical Ethical Committee of the Leiden University Medical Center (LUMC) and performed in accordance with the principles of the revised Declaration of Helsinki.[27] Written informed consent was obtained from all volunteers prior to participation.

## 2.2 | Study design

Participants were enrolled in a randomized, double-blinded, placebo-controlled cross-over study conducted from June 2017 to June 2018. The study consisted of three different interventions. During the first study visit, participants were exposed to an individualized water-cooling protocol to activate BAT, as previously described.[22] Estimated supraclavicular BAT volume and fat fraction were assessed with chemical shift-encoded magnetic resonance imaging (MRI). Only if BAT could be detected by MRI after cold exposure were participants then randomized to receive first either 200 mg mirabegron (Betmiga, Astellas BV, the Netherlands) or placebo in one oral dose. An overview of the study design is depicted in Figure S6. Before each study day, subjects fasted for 10 hours overnight and remained fasted until the end of the experiment.

### 2.2.1 | Study visit 1: cold exposure

During the first visit, a medical screening was performed to assess if participants met the inclusion criteria. In cases of eligibility, body composition was measured by bioelectrical impedance analysis (Bodystat 1500, Bodystat, UK). Precooling (thermoneutral conditions), a fasted blood sample was collected, and REE, lipid and glucose oxidation were measured via indirect calorimetry (Oxycon Pro, CareFusion, Germany) and cardiovascular variables (including heart rate and blood pressure) were assessed with Finapres Nova (Finapres Medical Systems BV, the Netherlands). Thereafter, a precooling MRI scan (3 T MRI, Philips Ingenia, Philips Healthcare, Best, the Netherlands) was performed to assess supraclavicular BAT fat fraction, transverse relaxation time (T2*) and estimated BAT volume using a three-dimensional six-point chemical shift-encoded gradient echo sequence, as described previously.[28] Next, 18 wireless iButtons were placed to monitor skin temperature (iButton, Maxim Integrated Products, CA, USA), and an individualized water cooling protocol was applied to activate BAT, as described previously.[22] After maximal non-shivering thermogenesis was reached, cold exposure continued for 60 more minutes. Thereafter, after ~ 2 hours, a blood sample was obtained and cold-induced REE, lipid and glucose oxidation were measured again. Lastly, a second MRI scan was performed to assess changes in supraclavicular BAT after cold exposure.

### 2.2.2 | Study visits 2 and 3: mirabegron and placebo treatment

During these study days, all measurements were performed under thermoneutral conditions. After measurement of body composition, a fasted blood sample was collected and REE, lipid and glucose oxidation, and cardiovascular variables were assessed. Next, mirabegron or placebo was ingested. One hour (t = 60 minutes), 2 hours (t = 120 minutes) and 3 hours (t = 180 minutes) after administration,

REE, lipid and glucose oxidation were assessed again. At 3.5 hours (t = 210 minutes), when reaching the maximum plasma concentration of mirabegron (i.e. $T_{max}$ ~3-4 hours), another blood sample was drawn and an MRI scan was performed to assess changes in supraclavicular BAT. Between study visits 1 and 2 there was a minimum wash-out period of 1 week, and between study visits 2 and 3 it was 2 weeks.

## 2.3 | Analyses

### 2.3.1 | Serum measurements

Commercially available enzymatic kits were used to measure serum concentrations of TG and total cholesterol (Roche Diagnostics, the Netherlands), HDL-cholesterol (HDL-C) (Roche Diagnostics), FFA (Wako Chemicals, Germany) and glucose (Instruchemie, the Netherlands). Insulin concentrations were measured using ELISA (Crystal Chem, IL, USA). LDL-cholesterol (LDL-C) was calculated using the Friedewald equation.[29]

### 2.3.2 | Serum lipidomic analysis by high performance liquid chromatography-mass spectrometry

Serum lipidomic analysis was performed, essentially as described previously.[30,31] The dataset was processed using an in-house developed metabolomics pipeline written in R programming language (http://www.r-project.org).

### 2.3.3 | Skin temperature

Eighteen wireless iButton temperature sensors were placed as adapted from 14 prescribed ISO-defined positions[32] (forehead, left chest, right abdomen, right thigh, right shinbone, right foot, back of the neck, right scapula, left lower back, left upper leg, right deltoideus, right forearm, right fingertip, and left supraclavicular) and four additional positions (left hand, left lower leg, left elbow, and right armpit).[33] Data were analysed using Temperatus software.[34] Armpit temperature was estimated and used as a proxy of core body temperature.[35] Supraclavicular skin temperature was estimated from an iButton placed above the left clavicula. Distal skin temperature was calculated as the average temperature of the left hand and right foot.[36] Proximal skin temperature was defined as the average of the iButtons on the chest, abdomen, scapula and lower back.[37]

### 2.3.4 | Indirect calorimetry

$VO_2$, and carbon dioxide production were determined every minute. Mean $VO_2$ and $VCO_2$, obtained by indirect calorimetry, were entered

into Weir's abbreviated equation (see below) to estimate energy expenditure, and REE was calculated as $VCO_2/VO_2$:

$$REE\,(kcal/min) = 3.941 \times VO_2\,(L/min) + 1.106 \times VCO_2\,(L/min).$$

Additionally, nutrient oxidation rates (i.e. carbohydrate and fat oxidation) were determined using Frayn equations.[38]

### 2.3.5 | MRI analysis

An in-house water-fat separation algorithm was used to reconstruct fat fraction maps, combined with a region-growing scheme to mitigate main field inhomogeneity effects.[39–42] Regions of interest encompassing the known location of the left supraclavicular BAT depot[43] were drawn manually by one observer (Figure S7). Registration was performed using the image registration software Elastix.[44,45] The average fat fraction, T2*, and estimated BAT volume of the supraclavicular adipose depot were computed for pre- and postcooling, postmirabegron and postplacebo scans. Only voxels with a fat fraction of 50%-100% were included for data analysis. One participant was excluded from all MRI analyses because of a failure to reconstruct the scan caused by excessive movement.

### 2.3.6 | Statistical analysis

Data were analysed using IBM SPSS Statistics for Windows version 22.0 (SPSS, Chicago, IL, USA). Figures were created by GraphPad Prism version 7.00 (GraphPad Software, La Jolla, CA, USA). Paired t-tests were used to study the effect of cold exposure, mirabegron and placebo treatments on serum lipids and skin temperature. Furthermore, paired t-tests were used to study the effect of cold on REE and nutrient oxidation, and two-way repeated measures ANOVA was applied to study the effect of placebo versus mirabegron on REE and nutrient oxidation. To study differences between interventions (cold exposure vs. mirabegron vs. placebo) in BAT MRI outcomes and the deltas (value after minus before intervention) of serum lipids and skin temperature, we performed one-way ANOVA with Bonferroni adjustments for post hoc comparisons. Moreover, to study changes in REE and nutrient oxidation over time and to assess differences between mirabegron and placebo treatments herein, we performed a two-way repeated measures ANOVA with the variables 'time' (0, 1, 2 and 3 hours) and 'treatment' (mirabegron or placebo) as within-subject factors. For the lipidomics data, mixed model analyses were used. P-values were adjusted for false rate of discovery (FDR) using the Benjamini-Hochberg procedure. All main analyses are presented per ethnicity (Europids vs. South Asians), as well as combined for both ethnicities as we did not observe interaction between ethnicity, treatment and metabolic outcome variables. $P < .05$ was considered statistically significant.

**TABLE 1** Participant characteristics

|  | Europids (n = 10) | South Asians (n = 10) |
|---|---|---|
| Age (years) | 22.9 (2.2) | 24.4 (3.1) |
| Height (m) | 1.86 (0.06) | 1.77 (0.05)** |
| Weight (kg) | 77.7 (5.9) | 71.5 (7.6) |
| Body mass index (kg/m$^2$) | 22.3 (1.1) | 22.7 (1.8) |
| Waist circumference (cm) | 82.1 (5.6) | 78.2 (5.2) |
| Hip circumference (cm) | 86.7 (4.7) | 86.1 (5.4) |
| Fat mass (%) | 12.9 (2.5) | 16.7 (3.7)* |
| Fat body mass (kg) | 10.1 (2.5) | 11.9 (3.2) |
| Fat-free mass (kg) | 67.6 (4.2) | 59.5 (6.3)** |
| Glucose (mmol/L) | 4.5 (0.4) | 4.6 (0.3) |
| Insulin (pg/mL) | 126 (59.1) | 203 (182.8) |
| Free fatty acids (mmol/L) | 0.43 (0..2) | 0.48 (0.1) |
| Triglycerides (mmol/L) | 0.79 (0.5) | 0.87 (0.7) |
| Total cholesterol (mmol/L) | 4.8 (1.5) | 6.0 (1.3) |
| HDL-cholesterol (mmol/L) | 1.4 (0.2) | 1.2 (0.3) |
| LDL-cholesterol (mmol/L) | 3.1 (1.3) | 4.3 (1.3) |

*Note:* Values are presented as mean (standard deviation). Unpaired t-tests were used for comparison between South Asians versus Europids. *$P < .05$; **$P < .01$.

## 3 | RESULTS

### 3.1 | Participant characteristics

Participant characteristics are summarized in Table 1. Europid and South Asian participants were equal with respect to age (24.4 ± 1.0 vs. 22.9 ± 0.7 years) and BMI (22.7 ± 0.6 vs. 22.3 ± 0.3 kg/m$^2$). South Asians were, however, shorter (1.77 ± 0.1 vs. 1.86 ± 0.02 m, $P < .01$), had a higher body fat percentage (16.7% ± 1.2% vs. 12.9% ± 0.8%, $P < .05$) and lower fat-free mass (59.5 ± 1.9 vs. 67.6 ± 1.3 kg, $P < .01$) in comparison with Europids. Basal fasting glucose, insulin and lipid levels were comparable between ethnicities, except for LDL-C levels, which tended to be higher in South Asians compared with Europids (4.3 ± 0.4 vs. 3.1 ± 0.4 mmol/L, $P = .051$).

### 3.2 | Mirabegron increases serum FFA and insulin levels

Because active BAT takes up lipids and glucose from the circulation, we first compared the effect of cold exposure and mirabegron on these serum variables in Europids and South Asians.

Two hours of cold exposure increased total cholesterol (TC) in Europids only (+16%, $P < .05$; Figure 1A). This was accompanied by an increase in HDL-C (+9%, $P < .05$) in Europids, an observation that also reached significance in South Asians (+11%, $P < .01$; Table S1). TG levels were not changed upon cold exposure (Figure 1B), while FFA levels were increased, but only in Europids (+61%, $P < .001$;
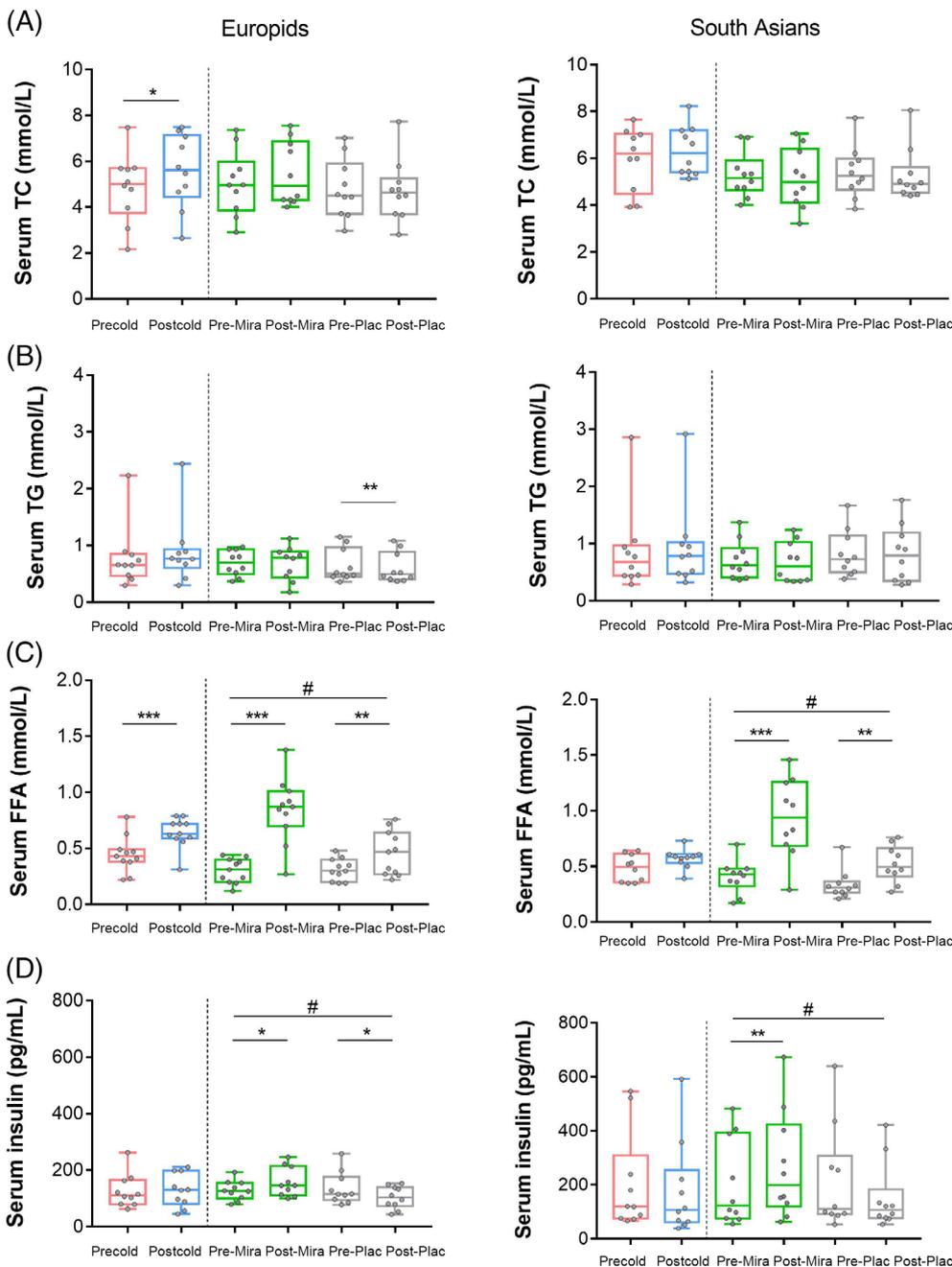
**FIGURE 1** Effect of cold exposure, mirabegron and placebo on serum lipids and insulin in Europids and south Asians. Serum was collected precold and postcold, mirabegron (Mira) or placebo (Plac) in Europids (*n* = 10) and south Asians (*n* = 10), and assayed for (A) total cholesterol (TC), (B) triglycerides (TG), (C) free fatty acids (FFA) and (D) insulin. Data are presented as means ±95% CI. Paired *t*-tests were used to assess the effect of the different treatments on serum variables. *P < .05; **P < .01; ***P < .001 before versus after intervention. One-way ANOVA was performed to study the deltas in time (after treatment minus before) between treatments. #P < .05 delta time between treatments

Figure 1C). Glucose, LDL-C (Table S1) and insulin (Figure 1D) levels were not affected by cold exposure in either ethnicity. There was no significant interaction between ethnicities and, therefore, we performed combined analyses of data from both ethnicities. Pooling of ethnicities showed that cold exposure significantly increased TC (Figure S1A), TG (Figure S1B) and FFA (Figure S1C).

One dose of mirabegron did not affect TC (Figure 1A), TG (Figure 1B), LDL-C or HDL-C (Table S1) in Europids or South Asians, or when both groups were combined in a single analysis (Figure S1 and Table S1). Mirabegron increased FFA levels in Europids (+214%, P < .001) and South Asians (+155%, P < .001) (Figure 1C). In addition, mirabegron similarly increased insulin levels in Europids (+23%,

P < .05) and South Asians (+38%, P < .01) (Figure 1D), without affecting glucose levels (Table S1).

## 3.3 | Mirabegron does not change the serum lipidome

To obtain a more comprehensive understanding of changes in the lipid profile induced by cold exposure and mirabegron, we performed a semi-targeted high performance liquid chromatography-mass spectrometry–based analysis of the lipidome in serum.

**FIGURE 2** Effect of cold exposure, mirabegron and placebo on serum lipidome in Europids and south Asians. Volcano plots showing lipidomics data in response to (A) cold exposure, (B) mirabegron or (C) the difference between mirabegron and placebo in Europids (n = 10) and south Asians (n = 10). Fold change represents the change of these lipids in comparison to the baseline (log (2)) (x-axis). P-value was corrected by the false rate of discovery (FDR). The horizontal dash line shows the level of significance (FDR-corrected P < .05). CE, cholesteryl ester; CER, ceramide; DG, diglyceride; LPC, lysophosphatidylcholine; PC-O, phosphatidylcholine etherphospholipid; (L)PE, (Lyso) phosphatidylethanolamine; PE-O, phosphatidylethanolamine etherphospholipid; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; SM4, sulfatide; TG, triglycerides. q-value represents P-value after FDR corrections
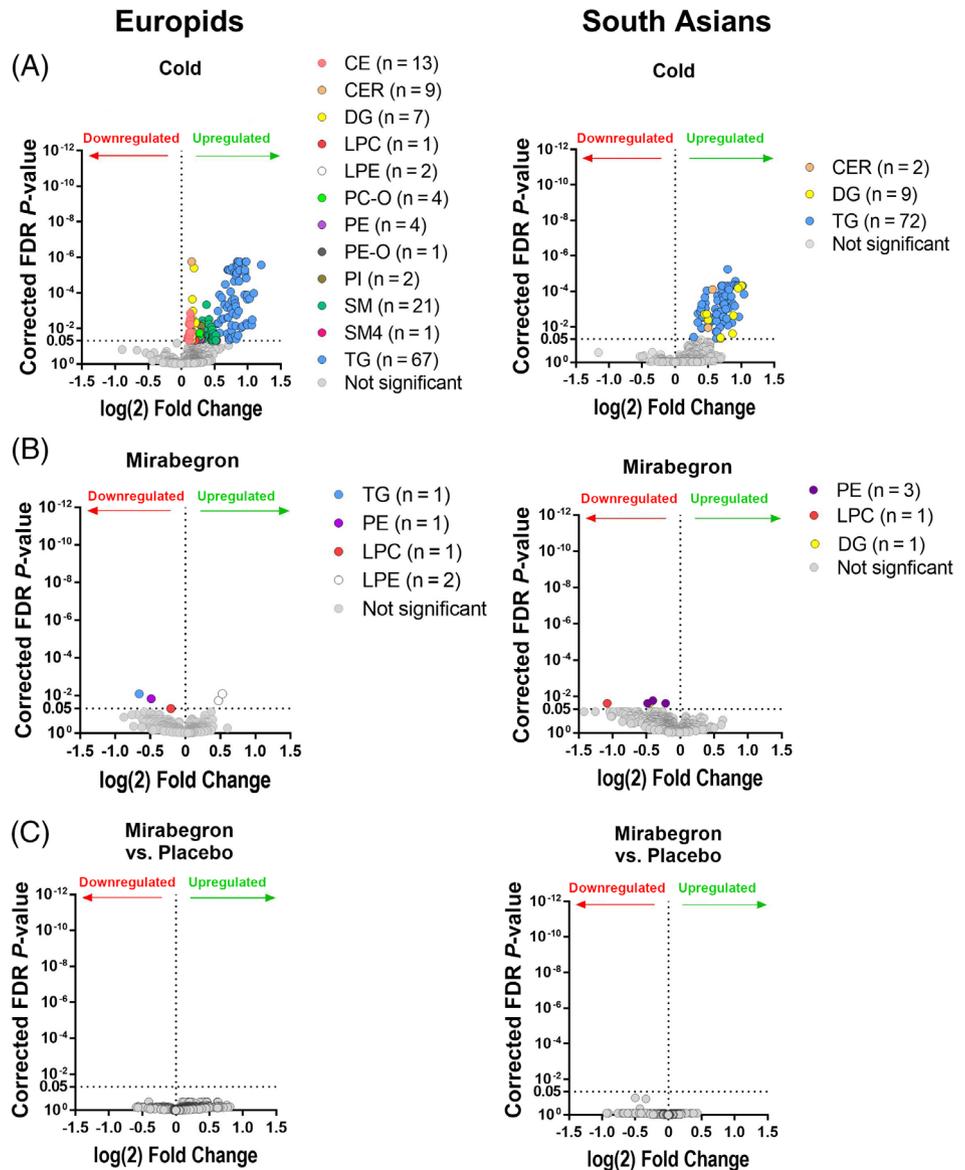


Cold exposure increased 132 and 83 out of ~1000 annotated lipid species in Europids and South Asians, respectively (Figure 2A). Of these increased lipid species, 67 (51%) and 72 (87%) were long-chain TG in Europids and South Asians, respectively. These changes were accompanied by increases in diglycerides in both ethnicities. Cold exposure also increased 21 sphingomyelins, 13 cholesteryl esters, nine ceramides and four phosphatidylethanolamines in Europids, whereas in South Asians only two ceramides were increased. Although this suggests an ethnicity-specific response to the cooling protocol, there were no statistically significant differences between Europids and South Asians upon cold exposure for any of the lipid species.

Interestingly, none of these cold-induced changes in the lipidome were observed in Europids or South Asians after treatment with mirabegron (Figure 2B) or placebo (not shown). In fact, mirabegron downregulated three lipid species in Europids and four in South Asians. In addition, there was no statistically significant difference

between mirabegron and placebo treatment for any of the lipid species in either ethnicity (Figure 2C), or when data of the individuals from both ethnicities were combined into a single analysis (Figure S2C).

## 3.4 | Mirabegron increases lipid oxidation

As BAT activation can influence energy expenditure and substrate use, we compared the effect of cold exposure and mirabegron on REE and lipid and glucose oxidation in Europids and South Asians.

Precooling REE was lower in South Asians compared with Europids (1347 ± 46 vs. 1563 ± 66 kcal/day, P < .05; Figure 3A), while lipid oxidation and carbohydrate oxidation were comparable. Of note, the ethnic differences in REE were no longer present after correction for lean body mass (data not shown). Cold exposure increased REE in
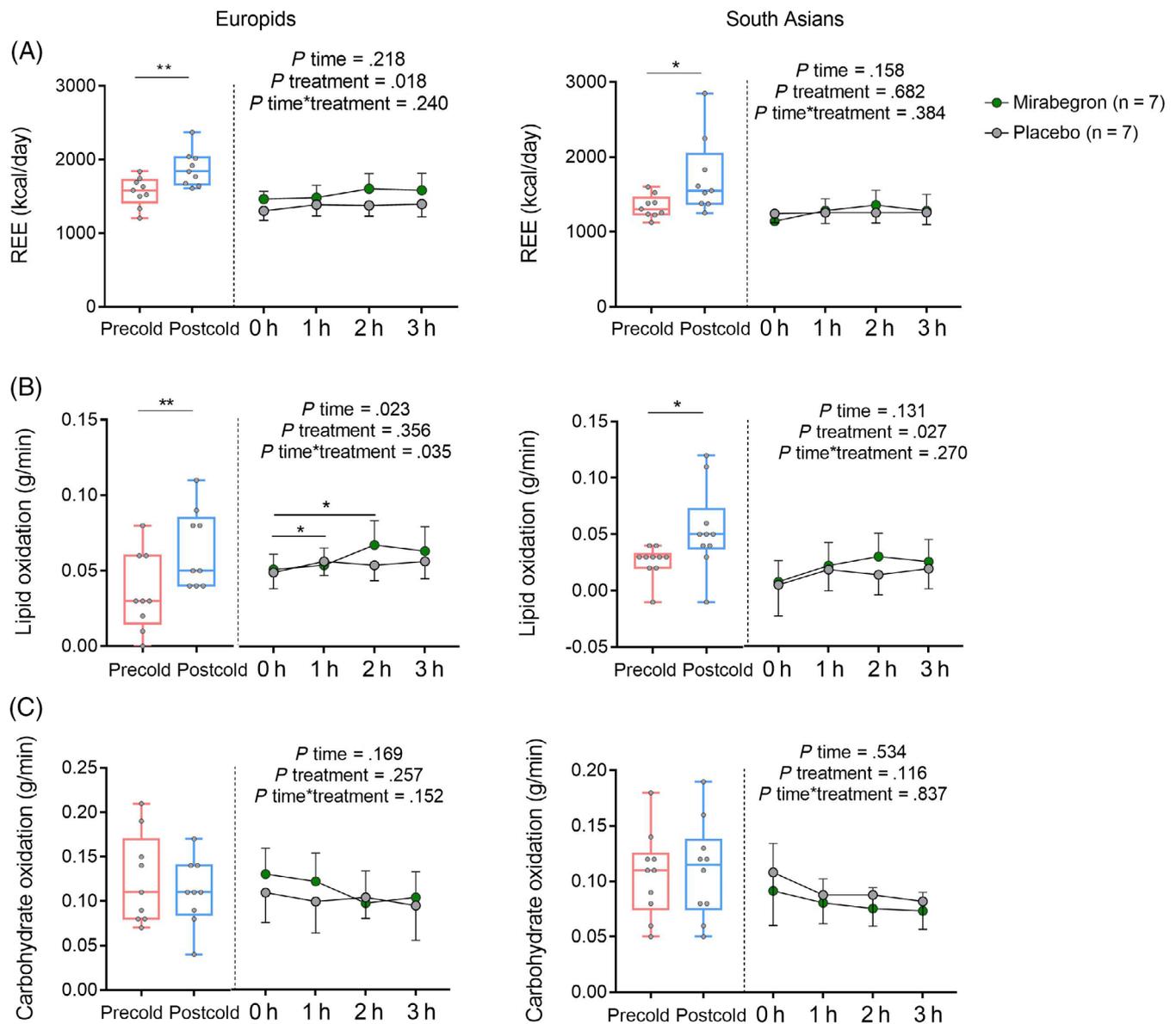
**FIGURE 3** Effect of cold exposure and mirabegron in comparison with placebo on resting energy expenditure (REE) and nutrient oxidation in Europids and south Asians. Precold (red boxes) and postcold (blue boxes) REE and nutrient oxidation in Europids (n = 10) and south Asians (n = 10). Paired t-tests were performed to study the effect of cold exposure. The effect of mirabegron or placebo on (A) REE, (B) lipid oxidation and (C) carbohydrate oxidation were studied by a repeated measures two-way ANOVA with 'time' (0, 1, 2 and 3 hours) and 'treatment' (mirabegron or placebo) as within-subject factors. The analyses were performed per ethnicity. P for time, P for treatment and P for time*treatment were obtained from the two-way ANOVA. Data are presented as mean ±95% CI. *P < .05; **P < .01

both Europids (+20%, P < .01) and South Asians (+29%, P < .05) (Figure 3A). In addition, cold exposure increased lipid oxidation in Europids (+114%, P < .01) and South Asians (+97%, P < .05) (Figure 3B), whereas carbohydrate oxidation remained unchanged in both ethnicities (Figure 3C). The increases in REE and lipid oxidation upon cold exposure were still observed when both groups were analysed together (Figure S3A and B).

Mirabegron treatment did not increase REE over time when compared with placebo in Europids or South Asians (Figure 3A). However, mirabegron did promote lipid oxidation in Europids when compared

with placebo (P for time*treatment = .035; Figure 3B), whereas this was not the case in South Asians (P for time*treatment = .270; Figure 3B). Mirabegron did not affect carbohydrate oxidation (Figure 3C).

Because two-way ANOVA with repeated measurements did not reveal an interaction between ethnicities in any of the tests (all P > .05), we performed combined analyses. These analyses showed that mirabegron significantly increased REE compared with placebo treatment, specifically in the second hour after treatment (Figure S3A). This was because of an increase in lipid oxidation (P for

**FIGURE 4** Effect of cold exposure, mirabegron and placebo on skin temperature in Europids and south Asians. Skin temperature was measured pre- and postcold, mirabegron (Mira) and placebo (Plac) in Europids (n = 10) and south Asians (n = 10). We directly measured (A) armpit and (B) supraclavicular skin temperatures, whereas (C) proximal and (D) distal skin temperatures were calculated following equations described in the supporting information. Data are presented as mean ±95% CI. Paired *t*-tests were used to evaluate the effect of the interventions. *P < .05, **P < .01, and ***P < .001 before versus after intervention. #P < .05 differences between the delta between treatments
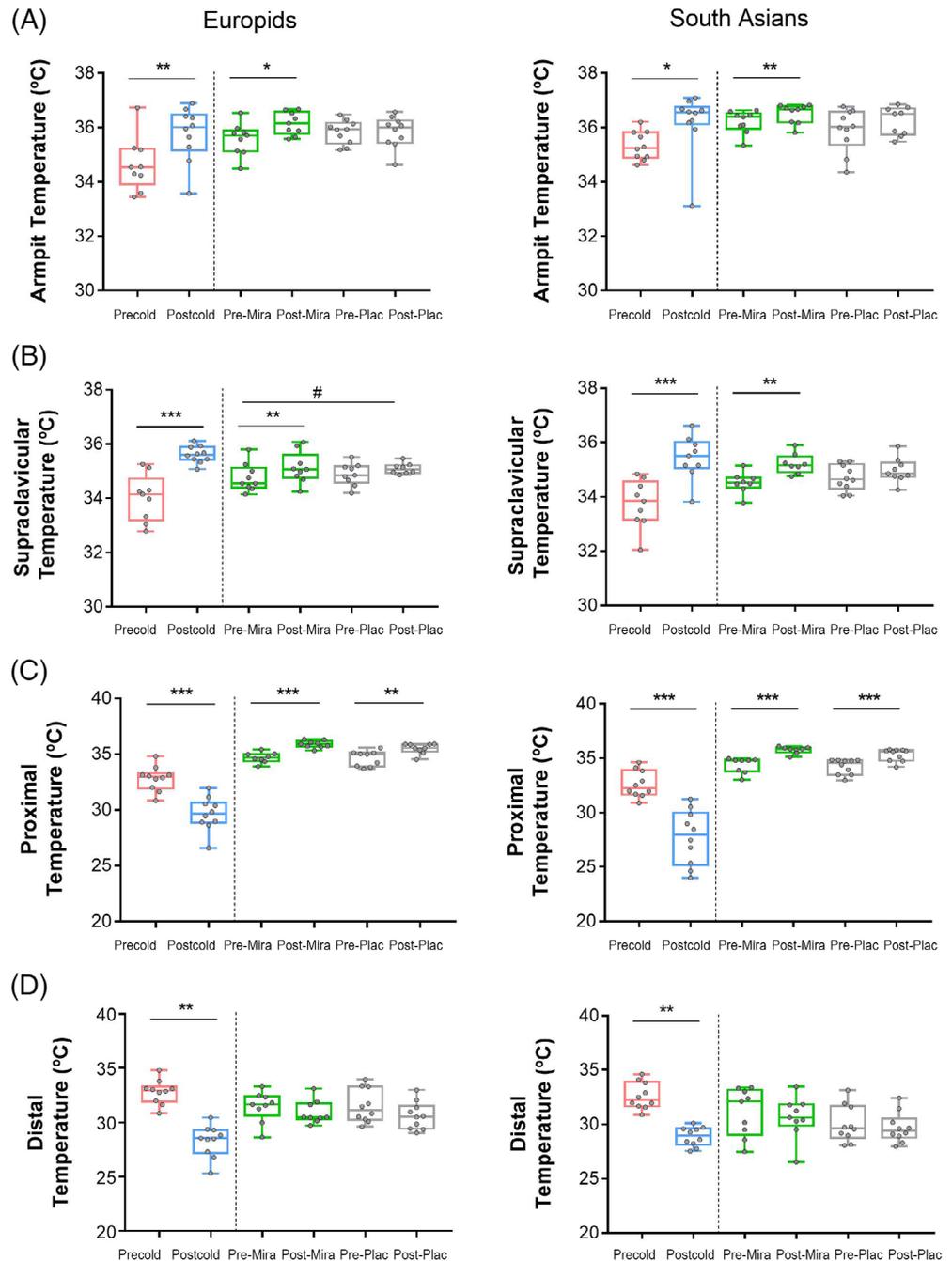


time*treatment < .001; Figure S3B), while carbohydrate oxidation was slightly decreased after 2 hours of treatment compared with baseline (P < .05; Figure S3C).

## 3.5 | Mirabegron increases supraclavicular skin temperature

The main function of BAT is heat production. Because supra-clavicular skin temperature positively associates with $^{18}$F-FDG uptake by BAT in young healthy lean men,[46] we compared the

effects of cold exposure and mirabegron on skin and core temperature in Europids and South Asians.

Cold exposure increased armpit skin temperature (as a proxy of core temperature) in Europids (+1.0°C, P < .01) and South Asians (+0.8°C, P < .05) (Figure 4A). Likewise, supraclavicular skin temperature was increased in Europids (+1.6°C, P < .001) and South Asians (+1.7°C, P < .001) (Figure 4B). Furthermore, as expected, cooling decreased proximal skin temperature in Europids (−3.2°C, P < .001) and South Asians (−4.9°C, P < .001) (Figure 4C) as well as distal skin temperature (−2.4°C, P < .01 and − 3.1°C, P < .01, respectively) (Figure 4D).

Mirabegron also increased armpit skin temperature in Europids (+0.6°C, $P < .05$) and South Asians (+0.3°C, $P < .01$) (Figure 4A). Furthermore, mirabegron increased supraclavicular skin temperature in both Europids (+0.4°C, $P < .05$) and South Asians (+0.7°C, $P < .01$) (Figure 4B). In contrast to cold exposure, mirabegron increased proximal skin temperature in Europids (+1.2°C, $P < .001$) and South Asians (+1.4°C, $P < .001$) (Figure 4C), without affecting distal skin temperature (Figure 4D). Of these measures, only the increase in supraclavicular skin temperature after mirabegron treatment in Europids was higher compared with placebo ($P < .05$; Figure 4B). Combining individuals of both ethnicities to perform a single analysis resulted in comparable results (Figure S4).

## 3.6 | Mirabegron reduces supraclavicular BAT fat fraction without affecting T2* or estimated BAT volume

Because BAT combusts intracellular lipids,[24] studying changes in fat fraction of the supraclavicular fat depot by MRI has been used as a read-out for BAT activity. Therefore, we compared the effect of cold exposure, mirabegron and placebo on BAT fat fraction, T2* and estimated BAT volume in Europids and South Asians. Hereby, T2* is defined as the effective transverse relaxation time which is influenced by both perfusion of oxygen-rich blood and the removal of deoxygenated blood in the tissue.[47] When BAT becomes activated, oxygen consumption increases because of enhanced metabolic activity and, at the same time, perfusion increases to keep up with this demand. Deoxygenated blood causes a local distortion of the magnetic field resulting in signal loss, and thereby a shorter T2*. However, increased perfusion leads to a longer T2* because of the presence of more blood, and therefore more oxyhaemoglobin. Thus, oxygen consumption leads to a decrease in T2*, whereas increased blood perfusion leads to the opposite effect.[27] Cold exposure lowered BAT fat fraction, both in Europids (−3.2%, $P < .001$; Figure 5A) and South Asians (−1.5%, $P < .05$). Cold did not affect fat fraction in the dorsocervical and deltoid subcutaneous adipose tissues, as well as in deltoid skeletal muscle (data not shown).

There was no difference in BAT fat fraction after mirabegron versus placebo treatment in Europids or South Asians. Also, compared with placebo, mirabegron did not affect fat fraction in the dorsocervical and deltoid subcutaneous adipose tissues, as well as in deltoid skeletal muscle (data not shown). Furthermore, while there was no effect of any of the treatments on BAT T2*, cold exposure lowered the estimated BAT volume in Europids only, probably as a result of lowered fat fraction (Figure 5B,C). When both ethnicities were combined in a single analysis, cold exposure still lowered BAT fat fraction (−2.3%, $P < .001$; Figure S5A) as well as estimated BAT volume (−1.3%, $P < .05$; Figure S5C). Of note, the average BAT fat fraction was lower after mirabegron versus placebo treatment (−1.4%, $P < .01$; Figure S5A). Furthermore, BAT T2* still remained unaltered after all treatments (Figure S5B).
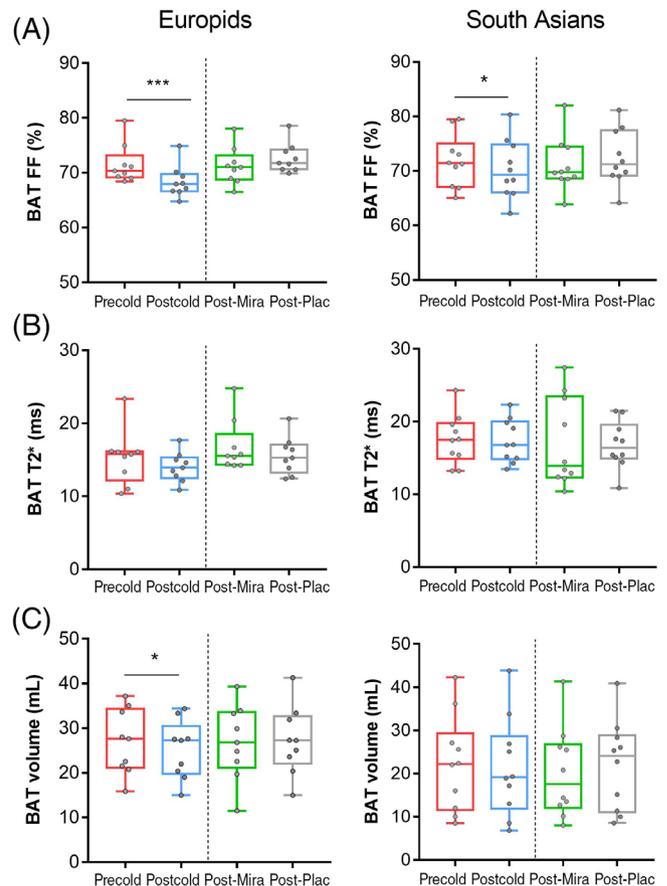


**FIGURE 5** Effect of cold exposure, mirabegron and placebo on brown adipose tissue (BAT) fat fraction (FF), T2* and estimated volume in Europids and south Asians. MRI was used to determine (A) BAT FF, (B) T2* and (C) estimated volume in Europids ($n = 10$) and south Asians ($n = 9$). Red boxes represent BAT-related outcomes before cold exposure and blue boxes represent BAT-related outcomes after cold exposure, green boxes after mirabegron (Post-Mira) and grey boxes after placebo (Post-Plac) treatment. All analyses were performed per ethnicity. One-way ANOVA was performed to study differences in BAT variables between treatments. *$P < 0.05$ and ***$P < .001$ between treatments. One south Asian was excluded from analyses because of movement in the MRI

## 3.7 | Mirabegron increases heart rate

Although mirabegron is a comparatively specific β3-AR agonist, it does cross-react with β1-AR and β2-AR. Because subtypes of β-AR are abundantly present on heart and blood vessels, we investigated the effects of mirabegron on heart rate and blood pressure. Cold exposure decreased heart rate in white Caucasians (−2 beats/minute, $P < .01$) and tended to decrease heart rate in South Asians (−1 beats/minute, $P = .10$) (Table S2). In addition, cooling increased systolic (+9%, $P < .05$) and diastolic (+22%, $P < .05$) blood pressure in South Asians only (Table S2). Mirabegron increased heart rate both in South Asians (+10 beats/minute, $P < .01$) and white Caucasians (+7 beats/minute, $P < .001$), while systolic or diastolic blood pressure were not significantly changed.

## 4 | DISCUSSION

Targeting BAT by cold exposure or adrenergic receptor agonism is considered a treatment strategy to combat cardiometabolic disease, which is more prevalent in South Asians compared with Europids. In the current study, we investigated the effect of targeting BAT by cold exposure and the β3-AR agonist mirabegron on the serum lipidome, REE, lipid oxidation, skin temperature variables and BAT fat fraction, T2* and estimated BAT volume in healthy lean South Asians versus Europids. We found that the response to cold and mirabegron on these variables was largely comparable between both ethnicities. We report that, in all subjects combined, both cold exposure and mirabegron increase serum FFA levels, lipid oxidation and supraclavicular skin temperature, while they decrease BAT fat fraction compared with placebo. Cold exposure, but not mirabegron treatment, induced changes in the serum lipidome, including the appearance of long-chain TG and diglycerides. This study supports the notion that both cold exposure and mirabegron may induce beneficial metabolic effects in Europid and South Asian subjects.

Because we included both South Asians and Europids in the current study, this gave us the opportunity to investigate whether the response to cold exposure and mirabegron would be different between ethnicities. We had reason to hypothesize this, because South Asians have a lower FFA response upon cold exposure[22] and higher circulating endocannabinoid levels compared with Europids,[21] suggesting they have lower cold-induced sympathetic outflow to BAT. Because mirabegron is believed to activate BAT directly via β-AR, we thus expected a more pronounced effect of mirabegron on REE and BAT fat fraction in South Asians compared with cold by circumventing sympathetic activation. Here, we confirmed a lower FFA response in South Asians upon cold exposure. However, counteracting our hypothesis, mirabegron enhanced lipid oxidation compared with placebo only significantly in Europids, and the responses of other metabolic variables to mirabegron were comparable between Europids and South Asians. It could still be possible that the extent to which noradrenalin is released from sympathetic nerve endings is lower in South Asians, contributing to a lower sympathetic stimulation of BAT. Alternatively, β3 independent pathways may contribute to cold-induced activation of BAT and these may differ between ethnicities. Clearly, future studies are needed to clarify whether there is a true difference in sympathetic output upon cooling between South Asians and Europids.

We also aimed to compare the effects of mirabegron with cold exposure on several metabolic variables, and showed that mirabegron increased FFA levels to a greater extent than cold exposure in both ethnicities. Although placebo treatment also increased FFA levels, suggesting an effect of prolonged fasting on serum FFA, the increase in FFA levels after cold exposure and mirabegron was larger than after placebo in both groups. A possible explanation for the more pronounced increase in FFA levels after mirabegron compared with cold exposure may be a higher relative effect of mirabegron on liberating FFA from white adipose tissue compared with stimulating FFA uptake or combustion (e.g. by BAT). To further investigate specific changes in the lipidome, we also performed lipidomic analysis. We observed in both ethnicities that cold exposure, but not mirabegron, increased levels of long-chain TG, as well as a set of diglycerides. This is suggestive of increased hepatic production of very low-density lipoprotein (VLDL)-TG, probably attributable to globally enhanced sympathetic outflow as induced by cold exposure, coupled to increased peripheral lipolysis (e.g. by BAT). Indeed, we previously showed that a comparable duration and mode of cooling increased serum concentration of large VLDL-TG particles accompanied by an increased mean size of VLDL particles, further supporting enhanced hepatic VLDL production.[48] The fact that the changes in lipidome mainly point towards increased hepatic VLDL production, probably induced by global sympathetic activation following cold exposure, may well explain the lack of effect of mirabegron on the lipidome.

It would be interesting to study the effect of mirabegron in combination with a treatment that further stimulates FFA combustion (e.g. by inducing a stronger activation of BAT) to reveal potentially beneficial effects on blood lipids in the short timeframe that was used in our study. However, it might be expected that after prolonged therapy, FFA liberation will ultimately be compensated by increased energy expenditure.

In addition, in contrast to cold exposure, we observed that a single dose of mirabegron increased serum insulin levels without affecting glucose levels. This is in line with the data of Cypess et al.,[26] who also showed increased insulin levels upon administration of the same dose of mirabegron in healthy lean volunteers. While this may be a very early sign of insulin resistance, the mirabegron-induced increase in FFA may also stimulate the pancreas to release insulin,[49] which has been reported as essential for efficient energy replenishment of activated BAT, at least in mice.[50] Alternatively, mirabegron may induce insulin release through acting on the β3-AR on the pancreas. Stimulation of β3-AR on blood vessels in the pancreas might induce local vasodilatation resulting in increased blood flow,[51] and thus increased the supply of glucose and FA to β-cells, thereby stimulating insulin release. Insulin stimulates the activity of lipoprotein lipase in adipose tissues.[52] In addition, insulin increases glucose uptake by tissues because of increased translocation of GLUT4 to the cell membrane. In this way, increased insulin levels could contribute to increased uptake of TG-derived FA and glucose from the circulation by BAT to facilitate intracellular combustion.[50] By contrast, two recent studies have shown that long-term treatment (4-12 weeks) with mirabegron improves insulin sensitivity in healthy, slightly overweight and obese subjects, possibly because of enhanced adiponectin levels and/or improved β cell function.[53,54] An interesting result of the current study was that, in contrast to cold exposure, mirabegron did not affect REE in Europids or in South Asians. A small increase in fat oxidation was only observed in Europids. Interestingly, this increase was found after 2 hours, while the $T_{max}$ of mirabegron is 3-4 hours. We can only speculate about the underlying cause. Possibly, the effect on fat oxidation occurs acutely, resulting in a quick peak, at least in the Europids. When both ethnicities were combined in a single analysis, mirabegron did increase REE. In the study by Cypess et al.,[26] a similar dose of

mirabegron did induce a significant increase in resting metabolic rate (+203 ±40 kcal/day). Our data support the notion that the applied dose of mirabegron (200 mg) is less efficient in activating BAT compared with cold exposure. Possibly, cold activates BAT via other mechanisms besides β adrenergic signalling, such as via FFA release.

We also observed an increase in supraclavicular skin temperature upon mirabegron treatment, which may reflect local heat production, possibly as a consequence of BAT activation.[46] Alternatively, this may be attributable to a direct effect of mirabegron on skin blood flow. Supporting increased BAT activation, we found a reduced BAT fat fraction upon mirabegron treatment in the combined group analysis. As was expected, we did not find an increase in the estimated BAT volume after acute cold exposure and mirabegron treatment. Such an increase may have been foreseen, if participants were acclimated to cold conditions or treated with mirabegron for a longer period, resulting in the recruitment of beige/brown adipocytes.[55] Instead, we found a reduction in the estimated BAT volume after cold exposure because of the exclusion of MRI voxels, for which the fat fraction fell below the segmentation threshold, as is more extensively described in our previous work.[27] On the contrary, the estimated BAT volume after mirabegron treatment remained unaltered, which is most probably attributable to the smaller effect compared with cold exposure. Cypess et al.[26] previously reported a massive increase in uptake of the glucose label ^{18}F-FDG by BAT as measured via positron emission tomography-computed tomography (PET-CT) scan after the same dose of mirabegron as used in the current study. Besides resulting from more active BAT, this increased ^{18}F-FDG uptake might also result from vasodilation within BAT caused by binding of mirabegron on β3-AR on the endothelium of arteries or because of the stimulation of other adrenergic receptors on blood vessels within BAT.[56,57] It would be of interest to further investigate the extent to which mirabegron activates BAT, also because of the lack of increase in REE, as mentioned above. Future studies should probably also investigate BAT activity with other imaging modalities and tracers, such as 11C-acetate to investigate the oxidative capacity of the tissue. A positive feature of our study is that we were able to analyse the effect of cold exposure and mirabegron on multiple variables associated with BAT in two different ethnicities. In addition, a placebo was used to discriminate between the effects of mirabegron treatment and effects induced by, among others, prolonged fasting. A limitation of the current study is that we measured BAT fat fraction at only one time point after cold exposure, mirabegron and placebo treatment. Because activated BAT also takes up lipids from the blood to restore intracellular lipid stores, we cannot exclude that this interfered with measurement of fat fraction as a proxy of BAT activity. This may thus result in an underestimation of the effect size of cold exposure and mirabegron on combustion of intracellular TG by BAT. For future studies, it would be preferable to combine fat fraction measurement by MRI with a tracer for lipid uptake by PET-CT scan.[58] Furthermore, because we only found significant effects on REE and fat fraction after combining both ethnicities, the study may have been underpowered for these variables. Because of the exploratory nature of the study we did not correct for multiple testing. Furthermore, we only investigated healthy lean men. Future studies should investigate if these results also apply to the general population, including women.

In conclusion, we have shown that South Asians and Europids have a comparable beneficial metabolic response to mirabegron and cold exposure. More specifically, both mirabegron and cold exposure increased FFA, lipid oxidation and supraclavicular skin temperature, while they decreased supraclavicular BAT fat fraction. Only cold exposure induced changes in the lipidome indicative of changes in VLDL-TG production and lipolysis. Future studies should aim at unravelling the relative effect of both treatments on BAT activity by using alternative tracers such as those that assess glucose and lipid uptake, or oxidative capacity.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## AUTHOR CONTRIBUTIONS

KJN and LGMJ designed the study, collected, analysed and interpreted the data, wrote the manuscript, and contributed to the discussion. MPB and JB collected, analysed and interpreted the data, contributed to the discussion and reviewed/edited the manuscript. ASDSM, JAvdE, LAO, OD, J-BvK, MvW, KB and BMT analysed the data, contributed to the discussion and reviewed/edited the manuscript. KB collected the data and reviewed/edited the manuscript. JRR, AGW, KWvD, FMV and TC contributed to interpretation of the data and reviewed/edited the manuscript. HEK designed the study, interpreted the data, contributed to the discussion and reviewed/edited the manuscript. IMJ, HEK, JFPB, MRB and PCNR were responsible for the overall supervision and designed the study, interpreted the data, contributed to the discussion, and reviewed/edited the manuscript. All authors approved the final version of the manuscript. KJN and LGMJ share first authorship. MRB and PCNR share senior authorship.

## DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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## SUPPORTING INFORMATION

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