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

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# ABSTRACT

## Aims

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.

## Material and Methods

Ten lean Dutch South Asian (age 18–30 years; body mass index (BMI) 18–25 kg/m$^2$) 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, whereas 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.

# 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 the South Asian, are particularly vulnerable to develop cardiometabolic disease. This is likely due, at least in part, to their disadvantageous metabolic profile, consisting of susceptibility to develop 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]. As a result, 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 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 to 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 to Europids [22]. Taken together, these data suggest that South Asians have a lower sympathetic outflow upon cold exposure compared to 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 focussed 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 might be a potent way 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 dys-

lipidemia 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 compare these in healthy lean South Asian versus Europid men.

# MATERIAL AND METHODS

For more details of methods, see the supporting information.

## 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.

## Study design

Participants were enrolled in a randomized, double-blinded, placebo-controlled cross-over study conducted between June 2017 and 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 MRI. Only if BAT could be detected on 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 **Fig. S6**. Before each study day, subjects were fasted for 10 hours overnight and remained fasted until the end of the experiment.

*Study visit 1: Cold exposure*

During the first visit, a medical screening was performed to assess if participants met the inclusion criteria. In case of eligibility, body composition was measured by bioelectrical impedance analysis (BIA; 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 parameters (including heart rate and blood pressure) were assessed with

Finapres Nova (Finapres Medical Systems BV, Netherlands). Thereafter, a precooling MRI scan (3T 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.

*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 parameters were assessed. Next, mirabegron or placebo was ingested. One hour (t=60 minutes), two hours (t=120 minutes), and three 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.* Tmax ~ 3–4 hours), another blood sample was drawn and an MRI scan was performed to assess changes in supraclavicular BAT. Between study visit 1 and 2 there was a minimum wash-out period of 1 week, and between study visit 2 and 3 it was 2 weeks.



## Analyses

*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 Inc., IL, USA). LDL-cholesterol (LDL-C) was calculated using the Friedewald equation [29].

*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 the R programming language (*http://www.r-project.org*).

*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, 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].

*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].

*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 (**Fig. 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 post-placebo scans. Only voxels with a fat fraction between 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.

*Statistical analysis*

Data were analysed using IBM SPSS Statistics for Windows version 22.0 (SPSS Inc, 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 *vs.* mirabegron on REE and nutrient oxidation. To study differences between interventions (cold exposure versus mirabegron versus 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 posthoc 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 since we did not observe interaction between ethnicity, treatment and metabolic outcome parameters. A *P*-value <0.05 was considered statistically significant.

# RESULTS

## 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<0.01), had a higher body fat percentage (16.7±1.2 *vs.* 12.9±0.8%, p<0.05) and lower fat free mass (59.5±1.9 *vs.* 67.6±1.3 kg, p<0.01) in comparison to Europids. Basal fasting glucose, insulin, and lipid levels were comparable between ethnicities, except for LDL-C levels that tended to be higher in South Asians compared to Europids (4.3±0.4 *vs.* 3.1±0.4 mmol/L, p=0.051).

**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) * |

**Table 1.** Participant characteristics (continued)

| | **Europids (*n*=10)** | **South Asians (*n*=10)** |
|---|---|---|
| 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) |

Values are presented as mean (standard deviation). Unpaired t-tests were used for comparison between South Asians vs. Europids. * $p<0.05$ and ** $p<0.01$. .

## 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%, $p<0.05$; **Fig. 1A**). This was accompanied by an increase in HDL-cholesterol (HDL-C) (+9%, $p<0.05$) in Europids, an observation that also reached significance in South Asians (+11%, $p<0.01$; **Table S1**). TG levels were not changed upon cold exposure (**Fig. 1B**), while FFA levels were increased, but only in Europids (+61%, $p<0.001$; **Fig. 1C**). Glucose, LDL-C (**Table S1**) and insulin (**Fig. 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 (**Fig. S1A**), TG (**Fig. S1B**) and FFA (**Fig. S1C**).

One dose of mirabegron did not affect TC (**Fig. 1A**), TG (**Fig. 1B**), LDL-C or HDL-C (**Table S1**) in Europids or South Asians, nor when both groups were combined in a single analysis (**Fig. S1** and **Table S1**). Mirabegron increased FFA levels in Europids (+214%, $p<0.001$) and South Asians (+155%, $p<0.001$) (**Fig. 1C**). In addition, mirabegron similarly increased insulin levels in Europids (+23%, $p<0.05$) and South Asians (+38%, $p<0.01$) (**Fig. 1D**), without affecting glucose levels (**Table S1**).

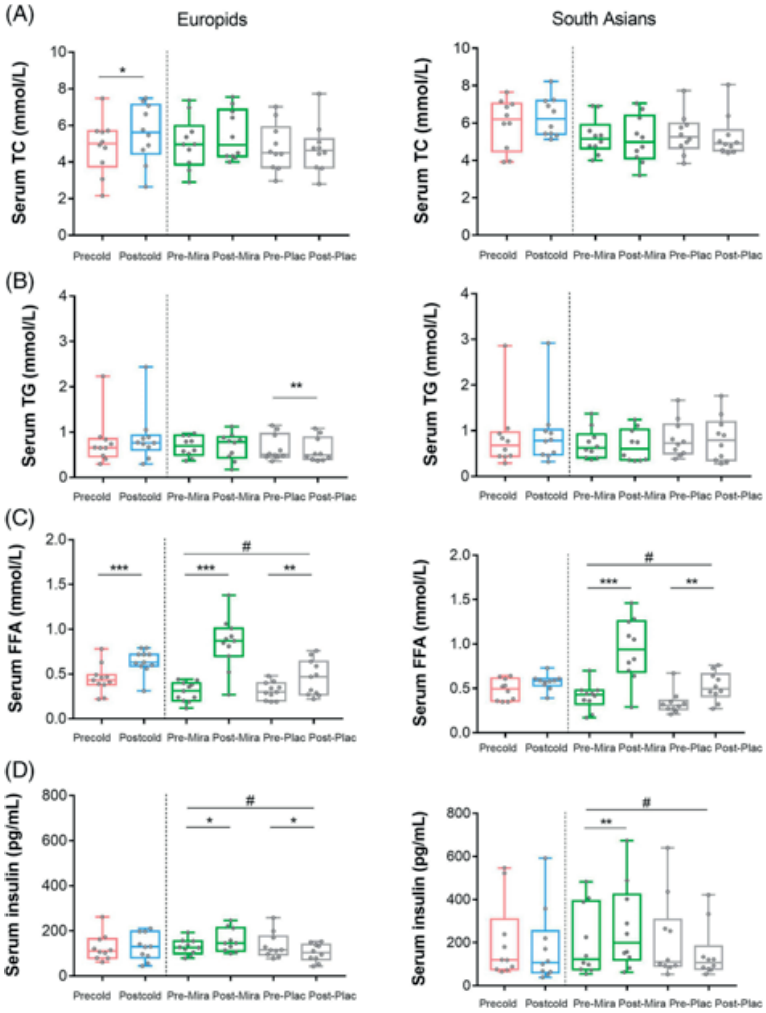


**Figure 1.** Effect of cold exposure, mirabegron and placebo on serum lipids and insulin in Europids and South Asians

Serum was collected pre-cold and post-cold, mirabegron (mira) or placebo (plac) in Europids (n=10) and South Asians (n=10), and assayed for total cholesterol (TC) (A), triglycerides (TG) (B), free fatty acids (FFA) (C), and insulin (D). Data are presented as means ± 95% CI. Paired t-tests were used to assess the effect of the different treatments on serum parameters * $p<0.05$, ** $p<0.01$, *** $p<0.001$ before vs. after intervention. One-way ANOVA was performed to study the delta's in time (after treatment minus before) between treatments. # $p<0.05$ delta time between treatments.

## 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 next performed a semi-targeted high performance liquid chromatography-mass spectrometry-based analysis of the lipidome in serum.

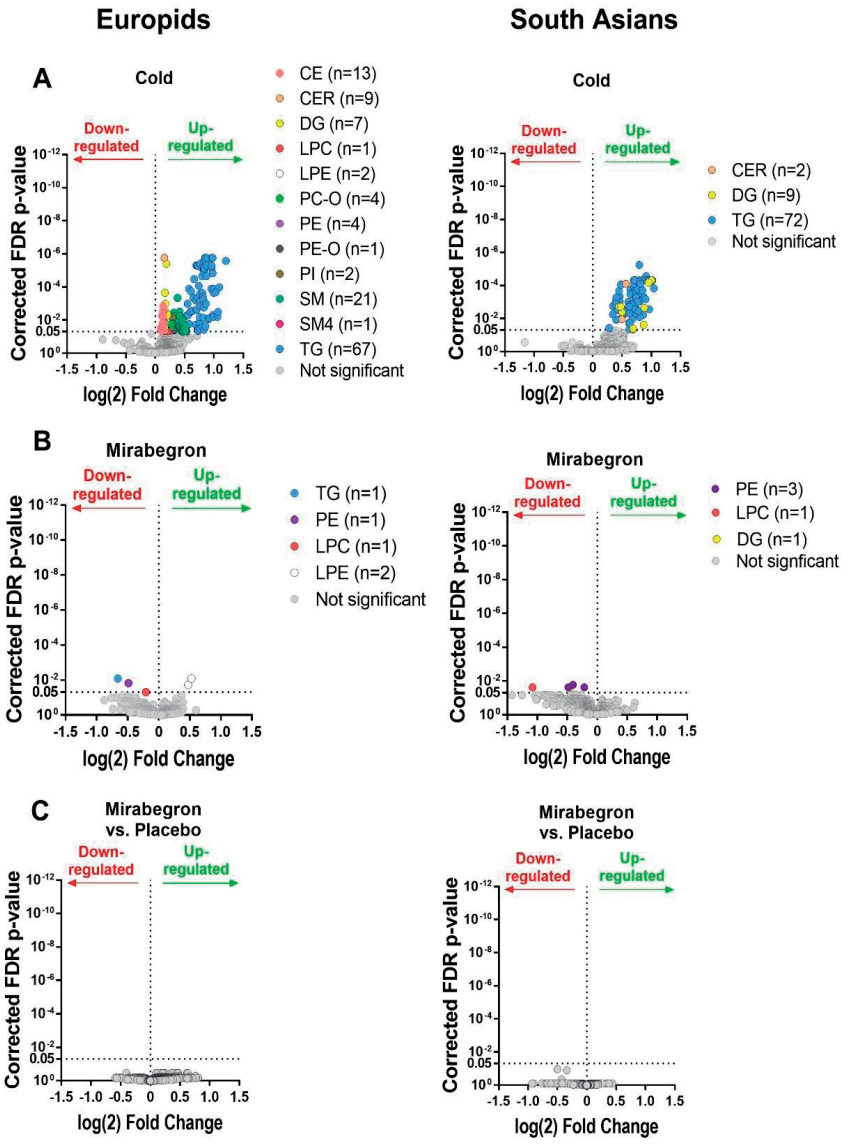


**Figure 2.** Effect of cold exposure, mirabegron and placebo on serum lipidome in Europids and South Asians
Volcano plots showing lipidomics data in response to cold exposure (A), mirabegron (B) or the difference between mirabegron and placebo (C) in Europids (n=10) and South Asians (n=10). Fold change 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<0.05). 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: triglyceride. 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 (**Fig. 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, 9 ceramides and 4 phosphatidylethanolamines in Europids, whereas in South Asians only 2 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 (**Fig. 2B**) or placebo (not shown). In fact, mirabegron downregulated 3 lipid species in Europids and 4 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 (**Fig 2C**), or when data of the individuals from both ethnicities were combined into a single analysis (**Fig. S2C**).

## 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, $p<0.05$; **Fig. 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 both Europids (+20%; $p<0.01$) and South Asians (+29%; $p<0.05$) (**Fig. 3A**). In addition, cold exposure increased lipid oxidation in Europids (+114%, $p<0.01$) and South Asians (+97%; $p<0.05$) (**Fig. 3B**), whereas carbohydrate oxidation remained unchanged in both ethnicities (**Fig. 3C**). The increases in REE and lipid oxidation upon cold exposure were still observed when both groups were analysed together (**Fig. S3A** and **3B**).

Mirabegron treatment did not increase REE over time when compared to placebo in Europids or South Asians (**Fig. 3A).** However, mirabegron did promote lipid oxidation in Europids when compared to placebo (p for time*treatment=0.035, **Fig. 3B**), whereas this was not the case in South Asians (p for time*treatment=0.270, **Fig. 3B**). Mirabegron did not affect carbohydrate oxidation (**Fig. 3C**).

Because two-way ANOVA with repeated measurements did not reveal interaction between ethnicities in any of the tests (all $p>0.05$), we performed combined analyses. These analyses showed that mirabegron significantly increased REE compared with placebo treatment, specifically in the second hour after treatment (**Fig. S3A**). This was

because of an increase in lipid oxidation (p for time*treatment <0.001, **Fig. S3B**), while carbohydrate oxidation was slightly decreased after 2 hours of treatment compared with baseline (p<0.05, **Fig. S3C**).

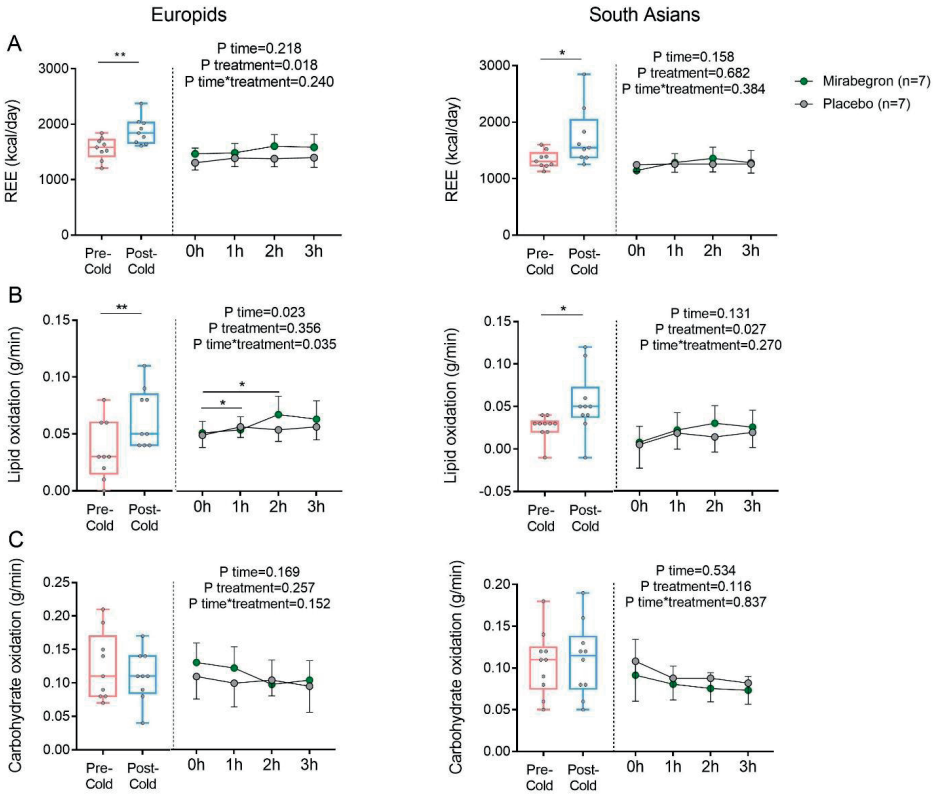


**Figure 3.** Effect of cold exposure and mirabegron in comparison with placebo on resting energy expenditure and nutrient oxidation in Europids and South Asians. Precold (red boxes) and postcold (blue boxes) REE and nutrient oxidation in Europids (n=10) and South Asians (n=10). Paired t-tests were performed to study the effect of cold exposure. The effect of mirabegron or placebo on REE (A), lipid oxidation (B) and carbohydrate oxidation (C) were studied by a repeated measures two-way ANOVA with 'time' (0, 1, 2 and 3 h) 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<0.05 and ** p<0.01.

## Mirabegron increases supraclavicular skin temperature

The main function of BAT is heat production. Because supraclavicular 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<0.01) and South Asians (+0.8°C, p<0.05) (**Fig. 4A**). Likewise, supraclavicular skin temperature was increased in Europids (+1.6°C, p<0.001) and South Asians (+1.7°C, p<0.001) (**Fig. 4B**). Furthermore, as expected, cooling decreased proximal skin temperature in Europids (-3.2°C, p<0.001) and South Asians (-4.9°C, p<0.001; **Fig 4C**) as well as distal skin temperature (-2.4°C, p<0.01 and -3.1°C, p<0.01, respectively) (**Fig. 4D**).

Mirabegron also increased armpit skin temperature in Europids (+0.6°C, p<0.05) and South Asians (+0.3°C, p<0.01) (**Fig. 4A**). Furthermore, mirabegron increased supraclavicular skin temperature in both Europids (+0.4°C, p<0.05) and South Asians (+0.7°C, p<0.01). (**Fig. 4B**). In contrast to cold exposure, mirabegron increased proximal skin temperature in Europids (+1.2°C, p<0.001) and South Asians (+1.4°C, p<0.001) (**Fig. 4C**), without affecting distal skin temperature (**Fig. 4D)**. Of these measures, only the increase in supraclavicular skin temperature after mirabegron treatment in Europids was higher when compared to placebo (p<0.05, **Fig. 4B**). Combining individuals of both ethnicities to perform a single analysis resulted in comparable results (**Fig. S4)**.

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

As 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 next 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 due to 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* due to the presence of more blood, and therefore more oxyhemoglobin. Thus, oxygen consumption leads to a decrease in T2*, whereas increased blood perfusion leads to an opposite effect [27]. Cold exposure lowered BAT fat fraction, both in Europids (-3.2%, p<0.001, **Fig. 5A)** and South Asians (-1.5%, p<0.05). 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, as compared to placebo, mirabegron dit 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 estimated BAT volume in Europids only, probably as a result of lowered fat fraction (**Fig. 5B** and **5C)**. When both

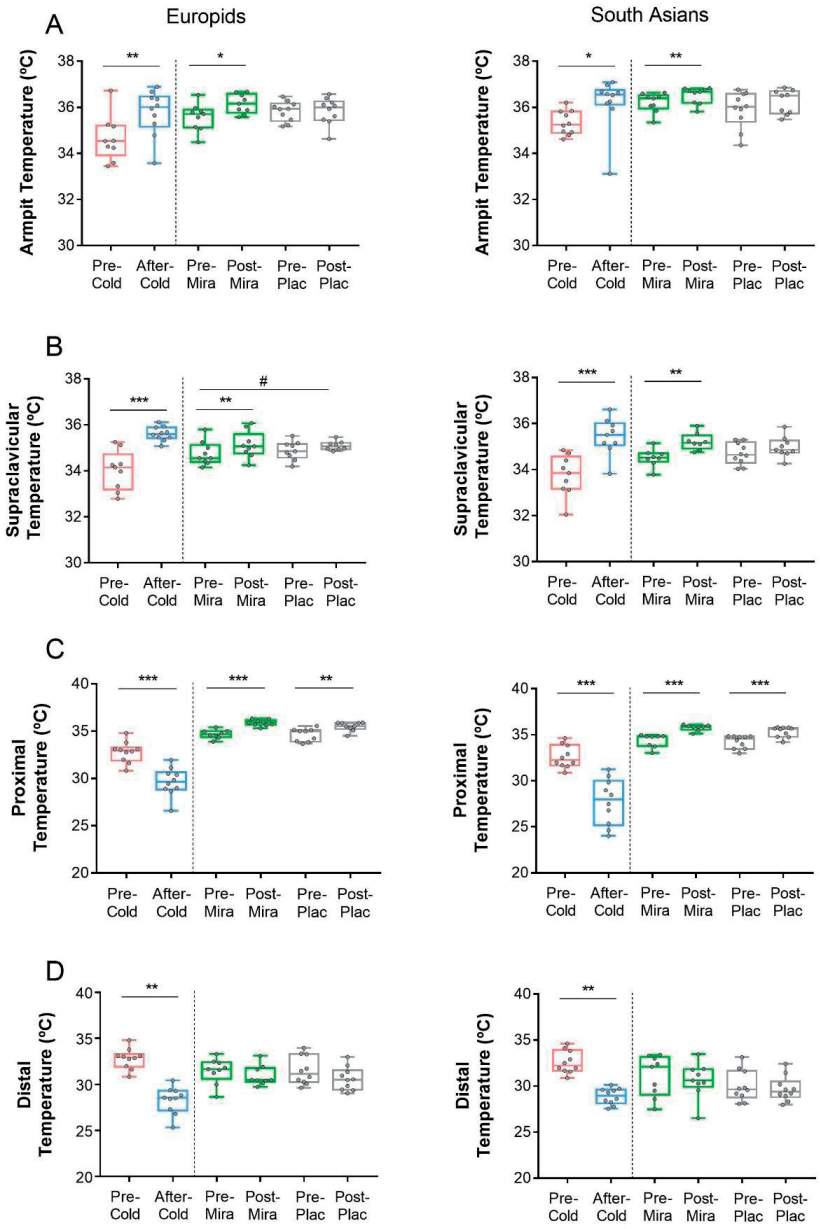


**Figure 4.** Effect of cold exposure, mirabegron and placebo on skin temperature in Europids and South Asians. Skin temperature was measured pre- and postcold, mirabegron (mira) and placebo (plac) in Europids (n=10) and South Asians (n=10). We directly measured armpit (A) and supraclavicular (B) skin temperatures, whereas proximal (C) and distal (D) 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<0.05, ** p<0.01, *** p<0.001 before vs. after intervention. # p<0.05 differences between the delta between treatments.

ethnicities were combined in a single analysis, cold exposure still lowered BAT fat fraction (-2.3%, p<0.001, **Fig S5A**) as well as the estimated BAT volume (-1.5%, p<0.05, **Fig S5C**). Of note, the average BAT fat fraction was lower after mirabegron versus placebo treatment (-1.4%, p<0.01, **Fig S5A**). Furthermore, BAT T2* still remained unaltered after all treatments (**Fig. S5B**).

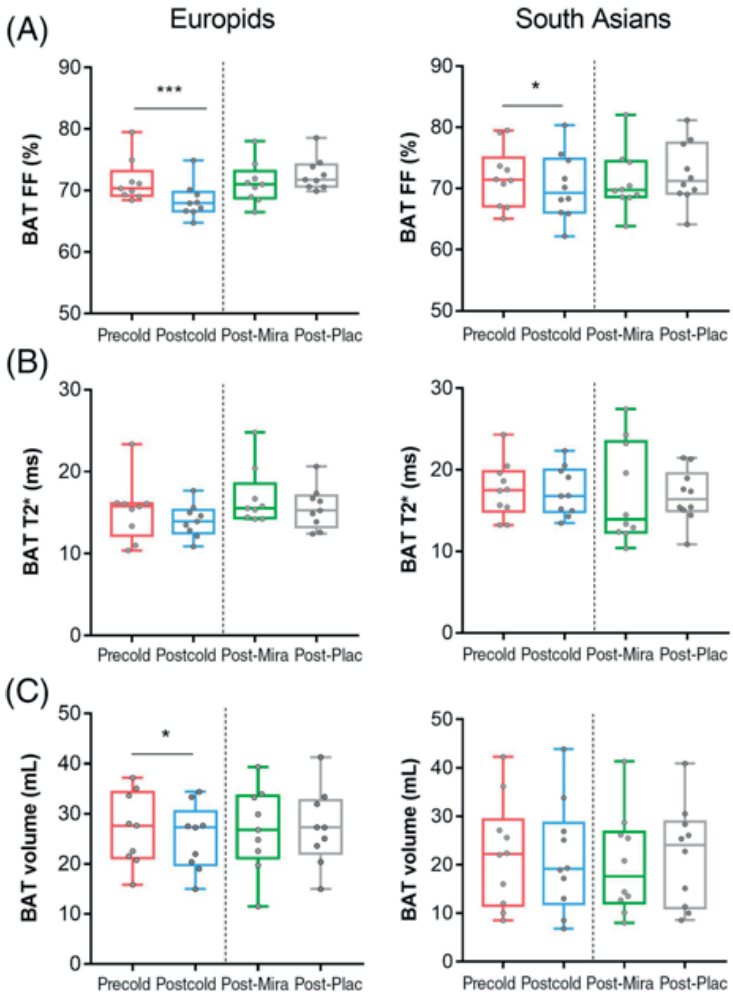


**Figure 5.** Effect of cold exposure, mirabegron and placebo on brown adipose tissue (BAT) fat fraction (FF), T2* and estimated volume in Europids and South Asians. MRI was used to determine BAT FF (A), T2* (B) and estimated volume (C) in Europids (n=10) and South Asians (n=9). Red boxes represent BAT-related 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<0.01 and *** p<0.001 between treatments. One South Asian was excluded from analyses because of movement in the MRI.

### Mirabegron increases heart rate

Although mirabegron is a comparatively specific β3-AR agonist, it does cross-react with β1-AR and β2-AR. Since subtypes of β-adrenergic receptors 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, $p<0.01$) and tended to decrease heart rate in South Asians (-1 beats/minute, $p=0.10$) (**Suppl. Table 2**). In addition, cooling increased systolic (+9%, $p<0.05$) and diastolic (+22%, $p<0.05$) blood pressure in South Asians only (**Suppl. Table 2**). Mirabegron increased heart rate both in South Asians (+10 beats/min, $p<0.01$) and white Caucasians (+7 beats/min, $p<0.001$), while systolic or diastolic blood pressure were not significantly changed.

## DISCUSSION

Targeting BAT by cold exposure or adrenergic receptor agonism is considered as 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 parameters 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 parameters 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 as compared to placebo. Cold exposure, but not mirabegron treatment, induced changes in the serum lipidome including appearance of long-chain triglycerides 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-ARs, 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 responses of other metabolic parameters 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 to 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 (VLDL-)TG, probably due 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 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 time frame 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 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 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 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 due to 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]. In 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 due to 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 resting energy expenditure in Europids nor in South Asians. A small increase in fat oxidation was observed in Europids only. 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 rather acute resulting in a quick peak, at least in the Europids. When both ethnicities were combined in a single analysis, mirabegron did increase resting energy expenditure. In a previous study of 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 mirabegron is less efficient in activating BAT as compared to cold exposure. Possibly, cold activates BAT via other mechanisms besides β adrenergic signaling, 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 due 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 due to 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 likely due to the smaller effect compared to 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 due to binding of mirabegron on β3-AR on the endothelium of arteries or due to stimulation of other adrenergic receptors on blood vessels within BAT [56,57]. It would be of interest to further investigate the extent by which mirabegron activates BAT, also because of the lack of increase in REE as mentioned above. Future studies should probably investigate BAT activity also with other imaging modalities and tracers, such as $^{11}$C-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 parameters 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, amongst 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 tracers that measure 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.



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# SUPPLEMENTAL APPENDIX

## Participants

Ten healthy, young (aged 18–30 years), lean (BMI 18–25 kg/m$^2$) Dutch South Asian men and ten age- and BMI-matched Europid men were included in the study. South Asian descent was defined as born in the Netherlands with both grandparents originating from the Indian subcontinent. Exclusion criteria were smoking, recent weight change (> 3 kg within the last 3 months), rigorous exercise, use of any medication known to influence glucose and/or lipid metabolism, BAT activity, cardiac function or QT interval time (*e.g.* beta blockers, thyroid medication, calcium channel blockers, monoamine oxidase inhibitors, or systemic corticosteroids), the presence of a chronic disease (including, but not limited to, T2D, thyroid disease, renal disease, or liver dysfunction), and/or contraindications for MRI. Contraindications for undergoing an MRI scan were the presence of non-MR safe metal implants or objects in the body (*i.e.* a pacemaker, neurostimulator, hydrocephalus or drug pump, non-removable hearing aid, or large recent tattoos), a history of claustrophobia, tinnitus, or hyperacusis. The study 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 [1]. Written informed consent was obtained from all volunteers prior to participation. Trial register clinicaltrials.gov number NCT03012113 (registration date: January 6, 2017).

## Study design

Participants were enrolled in a randomized, double-blinded, placebo-controlled crossover study consisting of three different interventions that were each performed on a different study day. The study was performed at the Leiden University Medical Center (Leiden, the Netherlands). During the first study visit, participants were exposed to an individualized water-cooling protocol to activate BAT as previously described [2]. Supraclavicular BAT volume and fat fraction were assessed with chemical-shift encoded MRI. As BAT combusts intracellular lipids [3], the intracellular fat fraction of BAT is expected to decrease upon activation. Therefore, the quantification of the fat fraction of BAT is considered to be a read-out for its activity. Only if BAT could be detected on MRI after cold exposure, participants continued with mirabegron and placebo treatment. Thereafter, participants were randomised to receive first either 200 mg mirabegron (Betmiga®, Astellas BV, the Netherlands) or placebo in one oral dose. After cold exposure, there was a wash-out period of at least one week, and the wash-out period between the mirabegron and placebo treatment was at least two weeks. An overview of the study design is depicted in **Fig. S6**. Before every study day, subjects were fasted for 10 hours overnight and remained fasted until the end of the experiment. A standardized dinner was consumed the evening before and participants wore standardized clothing consisting of a

boxer short and a thin pair of MRI-compatible trousers and hospital gown. The primary outcome measure of the study was BAT fat fraction. The secondary outcome measures were changes in REE, skin temperature and plasma lipidome. Based on a previous study assessing the effect of a cold intervention on BAT activity using MRI [4], we expected a decrease in TG content of 4% in BAT (SD = 1.7%). We expected the same effect for mirabegron and therefore, we considered a decrease in TG content of BAT by mirabegron of 4% as therapeutically relevant as this is as effective as cold exposure. Thus, with an SD of 1.7, $\alpha = 0.05$, $\beta = 20\%$ we needed 10 subjects per group.

*Randomisation and treatment allocation*

Mirabegron tablets were repacked into capsules by the LUMC pharmacy to resemble the placebo. All capsules were consecutively numbered for each subject according to the randomisation schedule. Researchers received the capsules in pre-packed idential looking bottles from the pharmacy. Thus, both participants and researches were blinded to treatment allocation. Randomisation was performed by the LUMC pharmacy. The random allocation sequence was generated by means of excel. Stratification was done on ethnicity and subjects were randomized in blocks of 4. In short, 2 groups were made of 12 numbers (number 1 up to 12 for South Asians and 13 up to 24 for Europids) and within each group of 12 numbers, 50% started with mirabegron and 50% with placebo. Allocation of randomization numbers was done in the order of inclusion.

*Study visit 1: Cold exposure*

During the first visit, a medical screening consisting of a medical questionnaire, measurement of height, weight and blood pressure, and a blood draw were performed to assess if participants met the inclusion criteria. In case of eligibility, the study day continued with insertion of an intravenous cannula and a measurement of body composition by bioelectrical impedance analysis (BIA; Bodystat 1500, Bodystat, UK). Pre-cooling (thermoneutral conditions), a fasted blood sample was collected, and resting energy expenditure (REE), lipid and glucose oxidation were measured via indirect calorimetry (Oxycon Pro, CareFusion, Germany) and cardiovascular parameters (including heart rate and blood pressure) were assessed with Finapres Nova (Finapres Medical Systems BV, Netherlands). Thereafter, a pre-cooling MRI scan (3T MRI, Philips Ingenia, Philips Healthcare, Best, the Netherlands) was performed to assess supraclavicular BAT fat fraction, transverse relaxation time (T2*) and volume using a three-dimensional six-point chemical-shift encoded gradient echo sequence as described before [5]. Participants were placed in supine position head-first in the scanner, with a 16-channel anterior array on the pelvis and their head in the 16-channel head and neck coil. After being placed on the scanner table, participants were asked to reach as far as possible with their fingers towards their feet and to relax their shoulders afterwards to ensure

reproducibility of subject positioning. The left supraclavicular BAT depot was assessed using a three-dimensional six-point chemical-shift encoded gradient-echo acquisition with the following parameters: repetition time TR=15 ms, first echo time TE=1.98 ms, echo time separation ΔTE=1.75 ms, flip angle=8°, field-of-view of 480 mm × 300 mm × 90 mm (Right-Left, Foot-Head, Anterior-Posterior), 1.1 mm isotropic resolution, four retrospective signal averages. 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 [2]). Participants were placed in a bed in semi-supine position between two water-perfused mattresses (BlanketRol® III, Cincinnati Sub-Zero Products, USA). The water temperature was set at 32°C and gradually decreased by 5°C every 10 minutes, until the participants started to shiver or until the minimum water temperature of 9°C was reached. Shivering was reported by the participants and confirmed visually by researchers. At that point, the water temperature was increased by 3°C to ensure maximal non-shivering thermogenesis. This cold exposure continued for 60 more minutes. Thereafter, after approximately 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.

*Study visit 2 and 3: Mirabegron and placebo treatment*

During these study days, all measurements were performed under thermoneutral conditions. After insertion of an intravenous cannula and measurement of body composition (again via BIA), a fasted blood sample was collected and REE, lipid and glucose oxidation, and cardiovascular parameters were assessed. Next, mirabegron or placebo was ingested. One hour (t=60 min), two hours (t=120 min), and three hours (t=180 min) after administration, REE, lipid and glucose oxidation were assessed again. At three and a half hours (t=210 min), when reaching the maximum plasma concentration of mirabegron (*i.e.* Tmax ~3–4 h), another blood sample was drawn and an MRI scan was performed to assess changes in supraclavicular BAT.

All participants were followed up up to 1 week after the last study visit.

## Analyses

*Serum measurements*

Commercially available enzymatic kits were used to measure serum concentrations of triglycerides and total cholesterol (Roche Diagnostics, the Netherlands), HDL-cholesterol (HDL-C) (Roche Diagnostics), free fatty acids (Wako Chemicals, Germany) and glucose (Instruchemie, the Netherlands). Insulin concentrations were measured using ELISA

(Crystal Chem Inc., IL, USA). LDL-cholesterol (LDL-C) was calculated using the Friedewald equation [6]. Data were analysed using SoftMaxPro 5.4.1 software.

*Serum lipidomic analysis*

Serum lipidomic analysis was performed essentially as described previously [7,8]. In short, lipids were extracted from 20 µL of serum and four HPLC-MS runs were performed on a reversed phase and a normal phase column and ions were detected by a Q Exactive Plus (Thermo Fisher Scientific, MA, USA) mass spectrometer in both the positive and negative ionisation mode yielding four raw data files per sample. The dataset was processed using an in-house developed metabolomics pipeline written in the R programming language (http://www.r-project.org) that identified and annotated features (peaks), performed isotope correction, and normalized data to the intensities of the added internal standards.

*Skin temperature*

18 wireless iButton temperature sensors were placed and adapted from 14 prescribed ISO-defined positions [9] (forehead, left chest, right abdomen, right thigh, right shinbone, right feet, back of the neck, right scapula, left lower back, left upper leg, right deltoideus, right forearm, right fingertip, left supraclavicular) and 4 additional positions (left hand, left lower leg, left elbow, and right armpit) [10]. Data were analysed using Temperatus® software [11]. Armpit temperature was estimated and used as a proxy of core body temperature [12]. 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 [13]. Proximal skin temperature was defined as the average of the iButtons on the chest, abdomen, scapula, and lower back [14].

*Indirect calorimetry*

Indirect calorimetry was performed in time-frames of 30 minutes. Participants were instructed to lie still and were not allowed to talk. $VO_2$ and carbon dioxide production were determined every minute. REE was calculated and substrate utilization was assessed by calculation of lipid and glucose oxidation after correction for protein oxidation, as described previously [15]. Both the thermoneutral and cold-induced measurement represent the average of the last twenty minutes.

*MRI analysis*

An in-house water-fat separation algorithm based on the known frequencies of the multi-peak fat spectrum and assuming mono-exponential effective transverse relaxation time (T2*) was used to reconstruct fat fraction maps, combined with a region-growing scheme to mitigate main field inhomogeneity effects [16-19]. Regions of interest

encompassing the known location of the left supraclavicular BAT depot [20] were drawn manually by one observer, exclusively on pre-cooling scans as described before (Abreu-Vieira 2020) (**Fig. S7)**. Registration was performed using the image registration software Elastix [21,22]. For calculating the deformation field, the first echoes of the pre- and post-cooling image stacks were co-registered using a three-dimensional B-spline transform with a 10×10×10 $mm^3$ grid, adaptive stochastic gradient descent with two resolutions for optimization and Mattes mutual information as the similarity measure (the registration parameter file can be downloaded from http://elastix.bigr.nl/wiki/index.php/Par0048). The calculated deformation field was subsequently used to map the pre-cooling region of interest to the coordinate space of the post-cooling images. Data analysis was performed within a 50-100% fat fraction interval. This segmentation range was applied for both the pre-cooling and post-cooling supraclavicular adipose tissue volumes. In this work, we set the lower FF threshold to 50%, which was based on our previously published work [5], wherein we analysed the effects of FF segmentation thresholds on MRI BAT-related outcomes and has been used by others in the field [23,24]. Post-mirabegron and post-placebo scans were analysed independently. The average fat fraction, T2* and total volume of the supraclavicular BAT depot were computed for pre-and post-cooling, post-mirabegron and post-placebo scans. One participant was excluded from all MRI analyses because of failure to reconstruct the scan due to excessive movement.



*Statistical analysis*

Data were analysed using IBM SPSS Statistics for Windows version 22.0 (SPSS Inc, 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 *vs.* mirabegron on REE and nutrient oxidation. In addition, 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 repeated measures ANOVA. 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 with ethnicity, intervention and study visit as fixed effects and subject specific deviances from the mean as random effects were used to assess the effects of mirabegron and cold exposure. In case the mixed model failed to converge, an ordinary linear model with only the fixed effects was 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). However, as we did not observe interaction between ethnicity, treatment and metabolic outcome parameters in any statistical test (all p>0.05), we also show all analyses combined for both ethnicities to increase the statistical power (**Fig. S1-S5**).A p-value<0.05 was considered statistically significant. Data are shown as mean and 95% CI, unless stated otherwise.

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**Table S1.** Effect of cold exposure, mirabegron and placebo on serum glucose, HDL-cholesterol and LDL-cholesterol in Europids, South Asians and both groups combined.

| Europids (n=10) | | | | | | |
|---|---|---|---|---|---|---|
| | Pre-cold | Post-cold | Pre-Mira | Post-Mira | Pre-Placebo | Post-Placebo |
| Glucose (mmol/L) | 4.5 (0.4) | 4.4 (0.4) | 4.5 (0.2) | 4.5 (0.2) | 4.4 (0.2) | 4.4 (0.4) |
| HDL-C (mmol/L) | 1.4 (0.2) | 1.5 (0.3)* | 1.4 (0.3) | 1.4 (0.3) | 1.4 (02) | 1.4 (0.3) |
| LDL-C (mmol/L) | 3.1 (1.3) | 3.6 (1.4) | 3.3 (1.3) | 3.7 (1.2) | 3.1 (1.2) | 3.1 (1.2) |
| South Asians (n=10) | | | | | | |
| | Pre-cold | Post-cold | Pre-Mira | Post-Mira | Pre-Placebo | Post-Placebo |
| Glucose (mmol/L) | 4.6 (0.3) | 4.5 (0.4) | 4.6 (0.3) | 4.7 (0.4) | 4.6 (0.5) | 4.5 (0.3) |
| HDL-C (mmol/L) | 1.2 (03) | 1.4 (0.4)** | 1.2 (0.2) | 1.2 (0.3) | 1.2 (0.3) | 1.3 (0.2) |
| LDL-C (mmol/L) | 4.3 (1.3) | 4.5 (1.0) | 3.8 (1.0) | 3.6 (1.3) | 3.8 (1.0) | 3.6 (0.1.0) |
| All (n=20) | | | | | | |
| | Pre-cold | Post-cold | Pre-Mira | Post-Mira | Pre-Placebo | Post-Placebo |
| Glucose (mmol/L) | 4.5 (0.3) | 4.4 (0.3) | 4.6 (0.2) | 4.6 (0.3) | 4.5 (0.4) | 4.5 (0.3) |
| HDL-C (mmol/L) | 1.3 (0.3) | 1.4 (0.3)*** | 1.3 (0.3) | 1.3 (0.3) | 1.3 (0.3) | 1.4 (0.3) |
| LDL-C (mmol/L) | 3.7 (1.4) | 4.1(1.3) | 3.5 (1.1) | 3.7 (1.2) | 3.5 (1.1) | 3.3 (1.1) |

HDL-C: HDL-cholesterol; LDL-C: LDL-cholesterol; Mira: mirabegron. Data are presented as mean and Standard Deviation. P-values were obtained from paired t-tests. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ after vs before treatment.



**Table S2.** Cardiovascular parameters.

| | Europids (n=10) | | | South Asians (n=10) | | |
|---|---|---|---|---|---|---|
| | Cooling | Mirabegron | Placebo | Cooling | Mirabegron | Placebo |
| Heart rate (bpm) | -2 ** | +7 *** | 0 | -1 | +10 ** | +2 |
| Diastolic BP (mmHg) | +13 | +6 | +4 | +13 * | +1 | +4 |
| Systolic BP (mmHg) | +16 | +4 | +7 * | +13 * | +9 | +6 |

Values are presented as delta (post- minus pre-intervention). Two-tailed paired (within ethnicities) was used for comparison before and after intervention. *** $p<0.001$, ** $p<0.01$, * $p<0.05$ difference compared to baseline measurement. BP, blood pressure

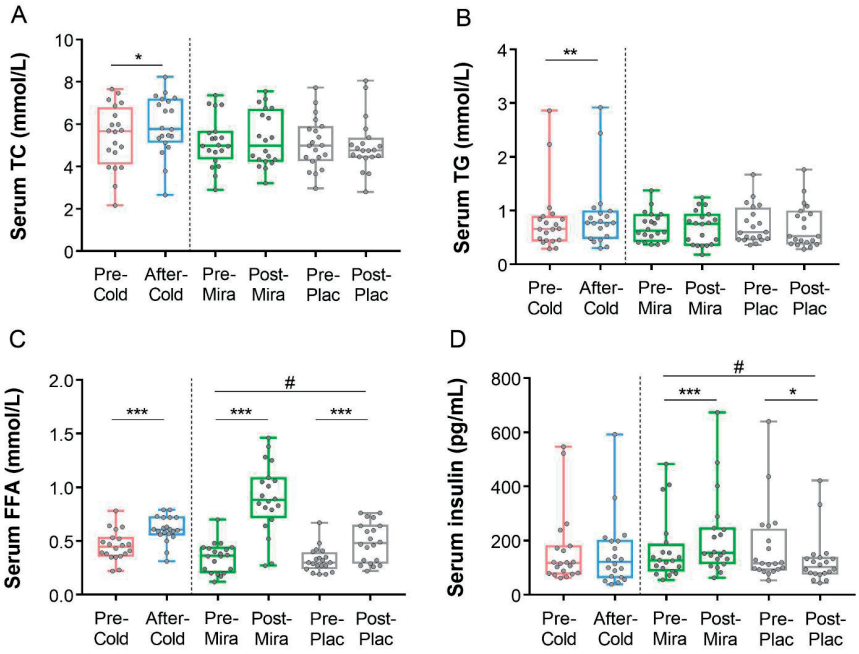


**Figure S1.** Effect of cold exposure, mirabegron and placebo on serum lipids and insulin in all subjects combined. Serum was collected pre- and post-cooling, mirabegron (mira) and placebo (plac) in all participants (n=20), and assayed for total cholesterol (TC) (A), triglycerides (TG) (B), free fatty acids (FFA) (C), and insulin (D). Data are presented as mean ± 95% CI. Paired t-tests were used to evaluate the effect of the interventions. * p<0.05, ** p<0.01, *** p<0.001 before vs. after intervention. One-way ANOVA was performed to study the delta's in time (after treatment minus before) between treatments. # p<0.05 delta time between treatments.

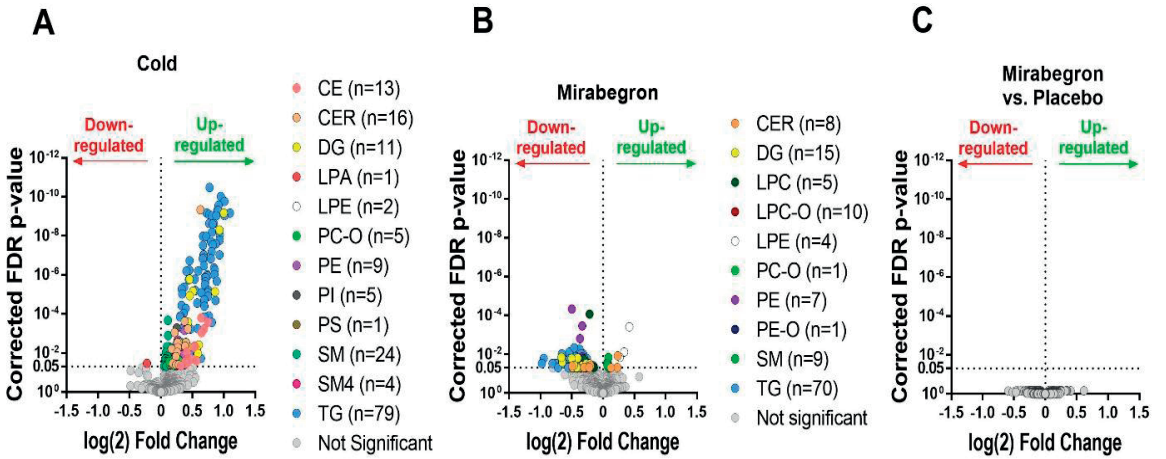


**Figure S2.** Effect of cold exposure, mirabegron and placebo on serum lipidome in all subjects combined. Volcano plots showing lipidomics data in response to cold exposure (A), mirabegron (B) or the difference between mirabegron and placebo (C) in all subjects combined. Fold change represents the change 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 $p<0.05$). CE: Cholesteryl ester; CER: Ceramide; DG: diglyceride; LPA: Lysophosphatidic acid; LPC: Lysophosphatidylcholine; (L)PC-O: (Lyso)phosphatidylcholine etherphospholipid; (L)PE: (Lyso)phosphatidylethanolamine; PE-O: phosphatidylethanolamine etherphospholipid; PI: Phosphatidylinositol; PS: Phosphatidylserine; SM: sphingomyelin; SM4: sulfatide; TG: triglyceride. q-value represents p-value after FDR corrections.

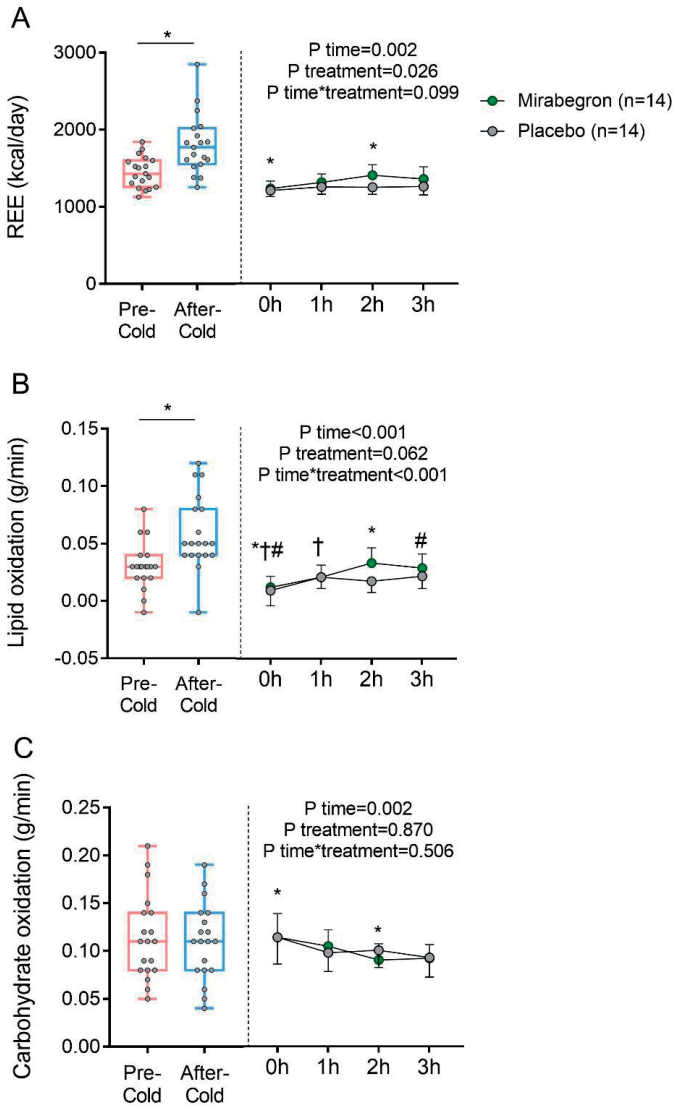


**Figure S3.** Effect of cold exposure and mirabegron in comparison to placebo on resting energy expenditure and nutrient oxidation in all subject combined**.** Pre-cold (red boxes) and post-cold (blue boxes) resting energy expenditure (REE) and nutrient oxidation in all subjects combined (n=20). Paired t-tests were performed to study the effect of cold exposure with The effects of mirabegron or placebo on REE (A), lipid oxidation (B) and carbohydrate oxidation (C) were studied by a repeated measures two-way ANOVA with 'time' (0, 1, 2 and 3 h) and 'treatment' (mirabegron or placebo) as within-subject factors. These analyses were performed with both ethnicities combined. 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<0.05.

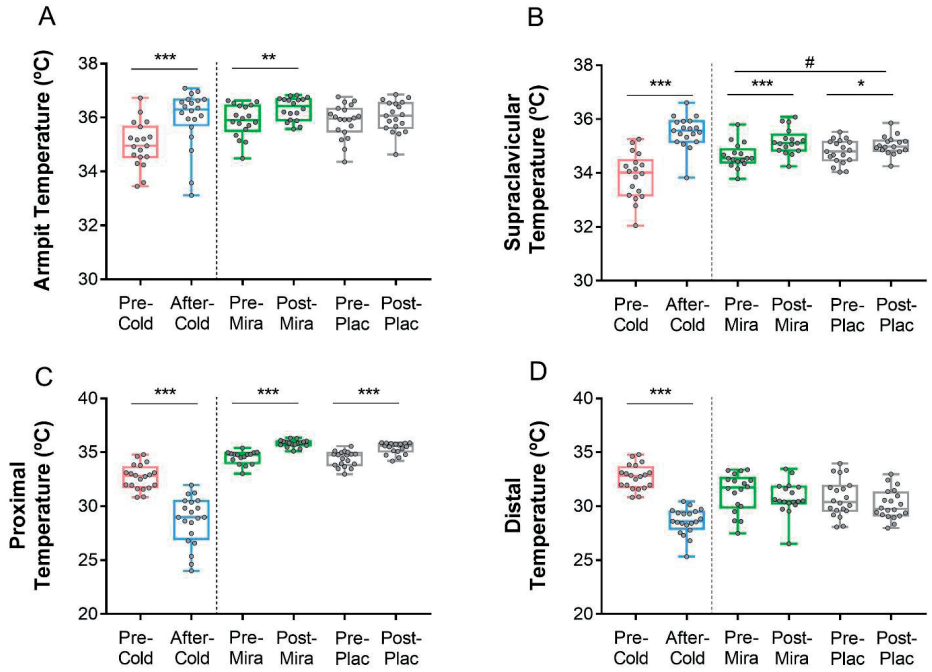


**Figure S4.** Effect of cold exposure, mirabegron and placebo on skin temperature in all subjects combined. Skin temperature was measured pre- and post-cold, mirabegron (mira) and placebo (plac) in all subjects combined (n=20). We directly measured armpit (A) and supraclavicular (B) skin temperatures, whereas proximal (C) and distal (D) skin temperatures were calculated following equations described in the ESM. Data are presented as mean ± 95% CI. Paired t-tests were used to evaluate the effect of the interventions. * p<0.05, ** p<0.01, *** p<0.001 before vs. after intervention. # p<0.05 differences between the delta between treatments.

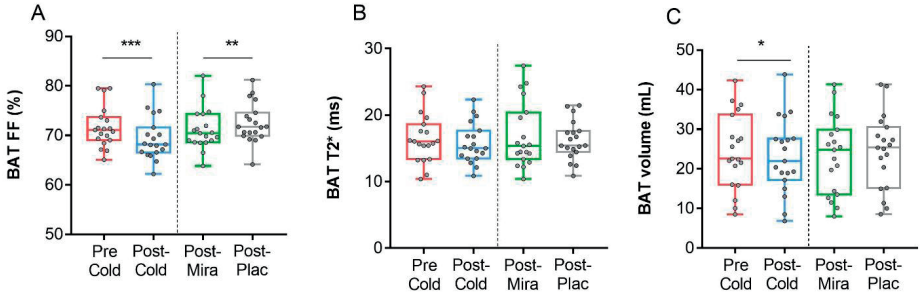


**Figure S5.** Effect of cold exposure, mirabegron and placebo on brown adipose tissue (BAT) fat fraction (FF), T2* and volume in all subjects combined. MRI was used to determine BAT FF (A), T2* (B) and volume (C). Red boxes represent BAT-related outcomes before cold exposure and blue boxes represent BAT-related outcomes after cold exposure, green boxes after mirabegron (mira) and grey boxes after placebo (plac) treatment. All analyses were performed with all available subject data (n=19). One-way ANOVA was performed to study differences in BAT parameters between treatments. ** p<0.01; *** p<0.001 between treatments. One South Asian was excluded from analyses due to movement in the MRI.

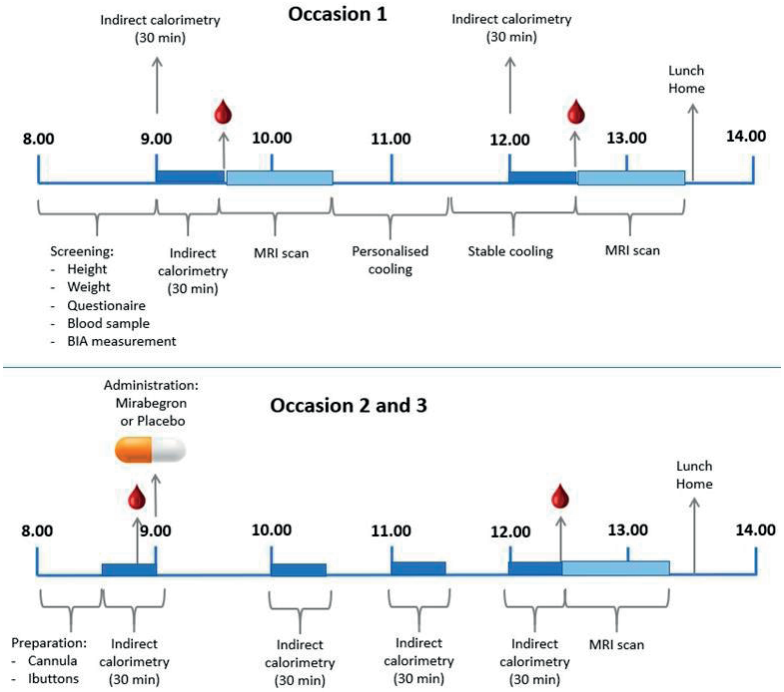


**Figure S6.** Overview of the study design**.** See text for explanation.

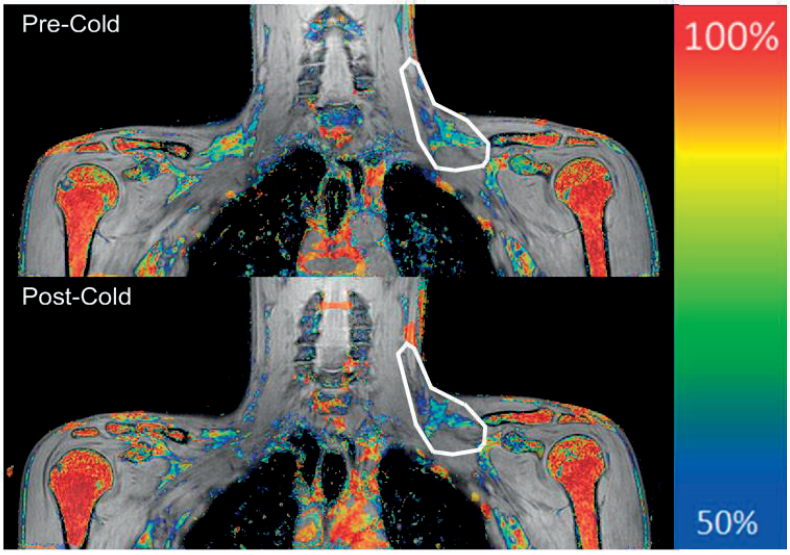


**Figure S7.** Region of interest drawn on the MRI scan**.** Example of supraclavicular region of interest drawn on MRI scan before and after cold exposure.