The K+ channel TASK1 modulates β-adrenergic response in brown adipose tissue through the mineralocorticoid receptor pathway

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Abstract

Brown adipose tissue (BAT) is essential for adaptive thermogenesis and dissipation of caloric excess through the activity of uncoupling protein (UCP)-1. BAT in humans is of great interest for the treatment of obesity and related diseases. In this study, the expression of Twik-related acid-sensitive K(+) channel (TASK)-1 [a pH-sensitive potassium channel encoded by the potassium channel, 2-pore domain, subfamily K, member 3 (Kcnk3) gene] correlated highly with Ucp1 expression in obese and cold-exposed mice. In addition, Task1-null mice, compared with their controls, became overweight, mainly because of an increase in white adipose tissue mass and BAT whitening. Task1(-/-)-mouse-derived brown adipocytes, compared with wild-type mouse-derived brown adipocytes, displayed an impaired β3-adrenergic receptor response that was characterized by a decrease in oxygen consumption, Ucp1 expression, and lipolysis. This phenotype was thought to be caused by an exacerbation of mineralocorticoid receptor (MR) signaling, given that it was mimicked by corticoids and reversed by an MR inhibitor. We concluded that the K(+) channel TASK1 [...]

Reference


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The K⁺ channel TASK1 modulates β-adrenergic response in brown adipose tissue through the mineralocorticoid receptor pathway

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ABSTRACT Brown adipose tissue (BAT) is essential for adaptive thermogenesis and dissipation of caloric excess through the activity of uncoupling protein (UCP)-1. BAT in humans is of great interest for the treatment of obesity and related diseases. In this study, the expression of Twik-related acid-sensitive K⁺ channel (TASK)-1 [a pH-sensitive potassium channel encoded by the potassium channel, 2-pore domain, subfamily K, member 3 (Kcnk3) gene] correlated highly with Ucp1 expression in obese and cold-exposed mice. In addition, Task1-null mice, compared with their controls, became overweight, mainly because of an increase in white adipose tissue mass and BAT whitening. Task1]+/−-mouse–derived brown adipocytes, compared with wild-type mouse–derived brown adipocytes, displayed an impaired β3-adrenergic receptor response that was characterized by a decrease in oxygen consumption, Ucp1 expression, and lipolysis. This phenotype was thought to be caused by an exacerbation of mineralocorticoid receptor (MR) signaling, given that it was mimicked by corticoids caused by an exacerbation of mineralocorticoid receptor expression, and lipolysis. This phenotype was thought to be the result of an increase in white adipose tissue through the mineralocorticoid receptor pathway. The K⁺ channel TASK1 modulates β-adrenergic response in brown adipose tissue through the mineralocorticoid receptor pathway.

Key Words: UCP1 · KCNK3 · thermogenesis · aldosterone · obesity

Obesity has reached epidemic proportions worldwide and constitutes a substantial risk factor for type 2 diabetes (T2D) and cardiovascular disease (1). An increase in body weight is accompanied by an increase in the mass of white adipose tissue (WAT) resulting from an imbalance in energy intake and energy expenditure. Regarding energy expenditure, brown adipose tissue (BAT) in rodents has long been known to be involved in adaptive (nonshivering) thermogenesis and to dissipate caloric excess through diet-induced thermogenesis (2). In contrast to early contentions, we are now aware that healthy human adults possess active BAT. BAT and brown adipocytes have recently been found in small amounts in healthy adult humans (3–9), and BAT activity correlates inversely with WAT mass (7, 8).

To achieve body temperature maintenance, BAT cells efficiently convert chemical energy, as carbohydrate and

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lipid sources, into heat. This thermogenic process is achieved by high mitochondrial content and increased respiration capacity, leading to an uncoupling between oxygen consumption and ATP synthesis caused by the expression of uncoupling protein (UCP)-1 (3, 10, 11). BAT is activated mainly by cold, and this function is mediated by the sympathetic nervous system and β-adrenergic receptors (ADRBs), particularly the β3 isoform (ADRB3), in rodents (12) and humans (13). In addition to the brown adipocytes located in BAT, WAT contains thermogenic fat cells, called brown-in-white (brite) or beige adipocytes, that burn fat and carbohydrates via non-shivering thermogenesis (3, 11, 14–17). Activation of these thermogenic cells is a promising target in the attempt to lower plasma glucose and fatty acid levels and diminish the risks of obesity and T2D. Therefore, the identification of factors governing mass and activity of human BAT would be of great interest in developing future treatments (18).

A gene expression profiling study identified the potassium channel, 2-pore domain, subfamily K, member 3 (KCNK3) as the only gene, besides UCP1, that was differentially expressed in BAT compared to WAT, in both humans and rodents (19). Twik-related acid-sensitive K+ channel (TASK)-1 (the protein product of the KCNK3 gene) is a member of the 2-pore domain K+ channels (K2P) family (20). This channel is sensitive to pH variations and is inhibited by extracellular acidification. So far, its known expression has been restricted to neuronal (central nervous system), muscular (heart, vascular smooth muscle), and endocrine (adrenal glands, pancreas) tissues (21). As a background potassium channel, it is involved in the control of resting membrane potential and as a result in the control of intracellular Ca2+ signaling and excitability (20). Task1-null mice displayed a peculiar phenotype of sex- and age-dependent primary hyperaldosteronism confined to adult female mice (22), caused by a surprising defect in functional zonation of the adrenal glands, resulting in permanent mineralocorticoid hormone production, independent of salt intake, and high blood pressure (23). Besides its effect on salt balance, aldosterone is also known to exert a detrimental effect on adipose tissue function and the development of metabolic dysfunctions (24). As an example, aldosterone has been demonstrated, via the activation of its receptor [mineralocorticoid receptor (MR)], to promote adipogenesis (25) and adipocyte hypertrophy and inflammation (26) and to be related to glucose metabolism disturbance (27). Recently, the KCNK3 gene locus has been linked to obesity in a large genome-wide association meta-analysis for body mass index (28). In addition, a recent report identified TASK1 as an important protein for brown adipocyte formation and function. However, the mechanisms that link this K+ channel to the adipocyte metabolism remain unknown (29).

In this study, we established a relationship between Task1 mRNA expression and control of body weight, through the characterization of its expression in cold-exposed and obese mice and through the identification of the mechanisms involving TASK1 in BAT activity. The knockout of Task1 in mice led to defective BAT and to an altered response of in vitro–derived brown adipocytes to β-adrenergic stimuli in terms of oxygen consumption, Ucp1 expression, and lipolysis. Finally, our results highlight a novel function for TASK1, as its regulation involves the MR, independent of circulating aldosterone levels.

MATERIALS AND METHODS

Reagents

Cell culture media and buffers were purchased from Lonza (Ozyme, Saint-Quentin en Yvelines, France); fetal bovine serum (FBS), insulin, and trypsin from Life Technologies–Invitrogen (Cergy Pontoise, France); and other reagents from Sigma-Aldrich Chimie (Saint-Quentin Fallavier, France). CL316243, dobutamine, terbutaline, carbonyl cyanide 4-(trifluoromethoxy)phenyl-hydrazone (FCCP), rotenone, antimycin A, and oligomycin were from Sigma-Aldrich Chimie.

Animals

The experiments were conducted in accordance with European regulations for the care and use of research animals and were approved by local experimentation committees (Ancona University: protocol 2007/No. 585, Ciepal Azur: protocol NCE-2012-57 and Geneva Cantonal Veterinary Office). The animals were maintained in constant temperatures (28±2°C or 22±2°C) and a 12:12 h light-dark cycle, with ad libitum access to standard chow diet and water.

Task1+/– mice were generated and backcrossed into a C57BL/6J (Janvier Labs, Le Genest-Saint-Issle, France) background. The Task1+/– mice used in this work were 10-wk-old male mice that did not display hyperaldosteronism when on a standard chow diet, whereas the female mice developed hyperaldosteronism (22).

ADRB agonist treatment consisted of a daily injection of CL316243 (1 mg/kg in saline solution, i.p.). Control mice were injected with vehicle.

For cold exposure, 10-wk-old female 129Sv mice (Charles River, L’Arbresle, France) were exposed for 10 d to 6°C (30). Nine-week-old B6.V-Lepob/J (Janvier Labs, Le Genest-Saint-Issle, France) background. The Task1+/– mice used in this work were 10-wk-old male mice that did not display hyperaldosteronism when on a standard chow diet, whereas the female mice developed hyperaldosteronism (22).

Stromal vascular fraction isolation and cell culture

Interscapular BAT, intra-abdominal (ia)WAT (epididymal), and subcutaneous (sc)WAT (inguinal) depots were sampled, washed in PBS, and minced. Adipose tissue samples were digested for 45 min at 37°C in DMEM containing 2 mg/ml collagenase A (Roche Diagnostics, Meylan, France) and 20 mg/ml bovine serum

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MR, mineralocorticoid receptor; OCR, oxygen consumption rate; Pña, perilipin A; qPCR, quantitative PCR; seWAT, subcutaneous white adipose tissue; Pref, preadipocyte factor; SVF, stromal vascular fraction; T2D, type 2 diabetes; TASK, Twik-related acid-sensitive K+ channel; UCP, uncoupling protein; WAT, white adipose tissue; WT, wild-type
albumin (Sigma-Aldrich Chimie). Lysate was successively filtered through 250, 100, and 27 μm nylon sheets and centrifuged for 5 min at 500 g. The pellet containing stromal vascular fraction (SVF) cells was subjected to a red blood cell lysis procedure. The SVF cells were plated and maintained in DMEM containing 10% FBS until confluence. Differentiation was induced in the same medium supplemented with 1 μM dexamethasone, 0.5 mM isobutylmethylxanthine, and 860 nM insulin for 2 d. The cells were then maintained for 7–10 d in the presence of 860 nM insulin for white adipogenesis and 860 nM insulin, 1 μM rosiglitazone, and 2 nM tri-iodothyronine for brown and beige adipogenesis. The medium was changed every other day.

Adipocyte fractions were obtained after centrifugation of the first 250 μm filtration at 500 g for 5 min, and floating adipocytes were sampled for molecular analysis.

**Isolation and analysis of RNA**

Total RNA was extracted with a TRI-Reagent kit (Euromedex, Souffelweyersheim, France), per the manufacturer’s instructions. Two micrograms of total RNA was subjected to RT-quantitative (q)PCR analysis (32) with SYBR qPCR premix Ex TaqII from Takara (Ozyme). The expression of selected genes was normalized to that of the 36b4 and Hprt housekeeping genes and then quantified by using the comparative ΔΔCt method. The oligonucleotide sequences are shown in Supplemental Table 1.

**Histology**

Histology was performed as has been described (33). Sections (4 μm) were dewaxed and treated in boiling citrate buffer (10 mM, pH 6.0). Sections were stained with hematoxylin and eosin.

**Figure 1.** Task1 mRNA expression in adipose tissue of obese, cold-exposed, and CL316243-treated mice. A) Task1 and Ucp1 mRNA levels were determined by RT-qPCR in interscapular BAT scWAT from 129/Sv male mice exposed to warmth (28°C) or cold (6°C). B) Task1 and Ucp1 mRNA expression were measured in 9-wk-old Ob/Ob and wild-type (WT) C57BL/6 mice housed at 22°C. C) Task1 or Ucp1 mRNA expression was evaluated in iaWAT and scWAT, interscapular BAT, heart, and liver from C57BL/6 male mice treated for 1 wk with the ADRB3 agonist, CL316243 (1 mg/kg/d) or with vehicle (Ve). Data are means ± SEM (n = 6 mice/group). *P < 0.05 vs. WT.
pH 6.0) for 6 min. Cooled sections were rinsed and then permeabilized in PBS 0.2% Triton X-100 at room temperature for 20 min. The sections were saturated in the same buffer containing 3% bovine serum albumen (BSA) for 30 min, incubated with anti-perilipin antibody (RDI-PROGP29; Research Diagnostic Inc., Flanders, NJ, USA) for 1 h, and tetramethylrhodamine isothiocyanate–coupled anti-guinea pig antibody for 45 min. Nuclear staining was performed with DAPI. UCP1 immunohistochemistry was performed according to the manufacturer’s instructions (LSAB+system-HRP; Dako, Les Ulis, France), using goat anti-UCP1 (clone C-17; Santa Cruz Biotechnology; Tebu-bio, Le Perray-en-Yvelines, France).

Visualization was performed with an Axiovert microscope, and images were captured with AxioVision software (Zeiss, Jena, Germany). Adipocyte diameter and lipid droplet area were measured with ImageJ software (National Institutes of Health, Bethesda, MD, USA). At least 40 adipocytes per section were measured, and 8 samples were analyzed per group.

Western blot analysis
Proteins were extracted from cells by using lysis buffer [25 mM Tris-Cl (pH 7.4), 100 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.5% Nonidet P40, and protease and phosphatase inhibitors; Roche Diagnostics, Meylan, France], and proteins from organs were extracted with a homogenizer (Ultra Turrax T25; IKA, Staufen, Germany) in the same buffer. Protein concentration was evaluated by bicinchoninic acid assay (Sigma-Aldrich Chimie). Five micrograms (for BAT) or 40 μg (for cells and scWAT) proteins were blotted in a basic SDS-PAGE protocol and processed (33). Primary antibodies were anti-UCP1 (662045; Calbiochem-Millipore, Molsheim, France), anti-β-tubulin (T4026; Sigma-Aldrich Chimie), and anti-TFAM (SC-28200; Santa Cruz Biotechnology). Detection was performed with horseradish peroxidase (HRP)–coupled secondary antibodies (Promega, Charbonnières-les-Bains, France) and Immobilon Western Chemiluminescent HRP Substrate (Calbiochem–Millipore). Chemiluminescence was detected with a ChemiDoc MP Imaging System and quantified with

Figure 2. Task1 mRNA expression in adipose tissue of cold-exposed mice and in isolated adipocytes. A) Fabp4, Task1, and Ucp1 mRNA expression was determined in the SVF and adipocyte (AF) fractions from scWAT and BAT of C57BL/6 mice. B) SVF cells from BAT, scWAT, and iaWAT were differentiated into brown, white, or brite adipocytes. At d 9, differentiated adipocytes were treated with CL316243 for 6 h and used for RNA analysis. Task1 and Ucp1 mRNA expression, as well as Plna (adipogenesis marker) and leptin (white adipocyte marker) were evaluated by RT-qPCR. Data are means ± SEM (n = 6 mice/group). aP < 0.05.
Triglyceride tissue content

BAT samples were weighed and disrupted in lysis buffer with a homogenizer (Ultra Turrax) at room temperature. Triglycerides (TGs) were immediately measured with a serum TG kit (T2449; Sigma-Aldrich Chimie), and free glycerol reagent (F6428; Sigma-Aldrich Chimie), according to the manufacturer’s instructions.

Lipolysis

Differentiated adipocytes were insulin-deprived overnight. Fresh medium was added, and the cells were immediately subjected to appropriate treatment (dobutamine, terbutaline, or CL316243) for 1 h 30 min. Sampled medium was used to measure glycerol release with a free glycerol reagent (Sigma-Aldrich Chimie), according to the manufacturer’s instructions. Results were normalized to protein amount.

Mitochondrial respiration analyses

SVF cells isolated from BAT or scWAT were seeded in 24-well plates and differentiated, as described herein. The oxygen consumption rate (OCR) of 8 d differentiated cells was determined with an XF24 Extracellular Flux Analyzer (Seahorse Bioscience, Proteigene, Saint Marcel, France). Isoproterenol (100 nM) was used to characterize β-adrenergic inducible respiration. Uncoupled and maximum OCR were determined with oligomycin (1.2 μM) and FCCP (1 μM). Rotenone (1 μM) and antimycin A (1 μM) were used to inhibit complex I- and III-dependent respiration, respectively. Parameters were measured for each well (34).

Statistical analyses

Data are expressed as means ± SEM and were analyzed with InStat software (GraphPad Software, La Jolla, CA, USA). Data were analyzed by Student’s t test or 1-way ANOVA followed by a Student-Newman-Keuls post hoc test. Differences that reached P < 0.05 were statistically significant.

RESULTS

Task1 and Ucp1 mRNA expression is correlated in thermogenic adipose tissues of WT and obese mice

The expression of Task1 was shown to be considerably higher in 129/Sv mouse BAT than in scWAT and to be somehow related to Ucp1 expression, the best known BAT activity marker. Cold exposure (6°C, 4 d) of the mice induced a significant increase in Task1 and Ucp1 mRNA expression in BAT, as compared to mice maintained at thermoneutrality (28°C) (Fig. 1A). A trend of increased Task1 expression, in parallel with that of Ucp1, was also noticed in scWAT of cold-exposed mice. In contrast, in the genetic mouse model of obesity (Ob/Ob mice), Task1 and Ucp1 mRNA expression was lower than in control mice, both in BAT and scWAT (Fig. 1B).

Comparison of Task1 mRNA expression in the different C57Bl/6J mouse adipose tissue depots also demonstrated a

![Figure 3](image-url)
clear overexpression in the interscapular BAT compared to the inguinal scWAT and the epididymal iaWAT (Fig. 1C). ADRB3 activation by CL316243 treatment of the mice resulted in a concomitant increase in the expression of Ucp1 and Task1 in all these tissues, further validating the association between Ucp1 and Task1 mRNA expression. As Ucp1 in scWAT is known to be expressed in brite adipocytes, it is tempting to speculate that brite adipocytes are also the site of Task1 expression after ADRB3 activation or cold exposure.

To confirm the expression of Task1 in thermogenesis-competent adipocytes (i.e., brown and brite adipocytes), we showed first that, like fatty acid–binding protein 4 (Fabp4) and Ucp1, Task1 expression was mainly confined to adipocytes, with very low expression in cells of the SVF (Fig. 2A). Moreover, we generated brown, brite, and white adipocytes in vitro by SVF cells derived from BAT, scWAT, and iaWAT (Fig. 2B). Differentiation was determined by using Ucp1 mRNA as a brown adipocyte marker, perilipin A (Plna) as a general adipogenic marker, and leptin as a white adipocyte marker (35). Task1 was expressed preferentially in brown and brite adipocytes, whereas only low levels were found in scWAT-derived white adipocytes and very low levels in iaWAT-derived white adipocytes. Finally, CL316243 treatment of adipocytes did not significantly affect Task1, Plna, and leptin mRNA expression in contrast to Ucp1.

**Task1 knockout promotes hypertrophic WAT and hyperlipidic BAT**

We used a knockout mouse model to examine whether TASK1 contributes to BAT activity. Task1-null (Task1−/−) male mice had a higher body weight compared to WT mice (Fig. 3A). The increased body weight was mainly due to increased WAT mass (Fig. 3B, C). In contrast to WAT, BAT weight was lower in Task1−/− than in WT mice (Fig. 3D). Histologic analysis showed that Task1−/− brown adipocytes had larger lipid droplets than did WT brown adipocytes (Fig. 4A). Indeed, quantification using perilipin immunostaining indicated larger lipid droplets in Task1−/− BAT, as displayed by their mean area and distribution (Fig. 4B). The increased lipid droplet size correlated with the TG content of BAT. Indeed, BAT from Task1−/− mice displayed a higher TG content per milligram of tissue (Fig. 4C). Whitening of Task1−/− BAT was not caused by a decrease in UCP1 protein and mRNA expression, as they were similar between WT and Task1−/− mice (Fig. 4D; Supplemental Fig. 1A). The same results were found for other BAT markers, including ADBR3, but with the exception of carnitine palmityltransferase-1m (Cpt1m) and cytochrome c mRNA, which showed higher expression in Task1−/− than in WT mice (Supplemental Fig. 1A). Among the adipocyte markers leptin, Fabp4,
Cd36, Pgc1b, and Pparg mRNA expression was higher in Task1−/− mice (Supplemental Fig. 1A), in agreement with the whitening of BAT (Fig. 3A). However, the expression of Pref1, an adipocyte progenitor marker, decreased significantly in Task1−/− mice, suggesting a decreased pool of adipocyte progenitors in Task1−/− mice BAT (Supplemental Fig. 1A). This observation was confirmed by analysis of the content of stromal cells (containing progenitors) of Task1−/− BAT, which was, indeed, lower than in BAT of WT mice (Fig. 4E). Of note, Pref1 expression was not modified in WATs, indicating that this feature was specific to BAT of Task1−/− mice (Supplemental Fig. 1B, C). Altogether, these results, in conjunction with the increased protein content found in Task1−/− BAT, indicated adipose tissue hyperplasia (Fig. 4F).

The increased WAT volume observed in Task1−/− mice was caused by an increase in the iaWAT mass (Fig. 5A), without any variation for scWAT (Fig. 5D). The increased iaWAT mass may have been associated with increased adipocyte size in Task1−/− vs. WT mice (Fig. 5B, C), which was not observed in scWAT (Fig. 5E, F). The analysis of adipocyte size distribution showed an increased frequency of larger adipocytes (>100 μm) in iaWAT of Task1−/− mice (Fig. 5C). Analysis of classic adipogenic markers in scWAT and iaWAT showed no significant alteration (Supplemental Fig. 1B, C) in contrast to BAT (Supplemental Fig. 1A).

**Task1 knockout hampers the ADRB3 stimulation**

As the main function of brown adipocytes is nonshivering thermogenesis mediated by catecholamine signaling, we analyzed the role of TASK1 after ADRB3 agonist treatment. Histologic analysis showed that BAT was activated by CL316243 treatment in both genotypes and was associated with a decreased size and number of lipid droplets (Fig. 6A, B). This activation was partially impaired in Task1−/− mice, as more large lipid droplets were found, associated with less UCP1 protein in this group, as revealed by immunostaining and Western blot analysis (Fig. 6B, C). Larger lipid droplets correlated with a higher TG content in Task1−/− BAT, even after CL316243 stimulation (Fig. 6D). The proportion of TG used by the tissue after CL316243 stimulation was also lower, with a 37.0 ± 4.1% decrease in WT BAT compared with only 20.1 ± 8.2% in Task1−/− BAT. In agreement with these observations, expression of brown adipocyte markers was significantly lower in the BAT of CL316243-treated Task1−/− mice than in WT (Fig. 6E).

Similar observations were obtained for scWAT, a tissue prone to brite adipocyte recruitment upon adrenergic stimulation. Indeed, CL316243-activated scWAT showed an attenuated browning phenomenon in Task1−/− compared to WT mice at the histologic, UCP1 protein, and gene expression levels (Fig. 7A–C).

**Task1 knockout alters oxygen consumption and the β-adrenergic response**

Task1 mRNA expression was confined to mature adipocytes (Fig. 2A), and its expression increased during brown, brite, and white adipocyte differentiation in mouse primary cultures (Supplemental Fig. 2C–F). However, Task1 deficiency did not affect brown adipocyte differentiation. Indeed, cell morphology and most of the brown adipocyte marker expression in SVF-derived adipocytes were similar in both genotypes (Supplemental Fig. 2A, C). Nevertheless, Ucp1 expression was slightly decreased in Task1−/− BAT-derived brown adipocytes (Supplemental Fig. 2C), pointing out a potential defect in mitochondrial function. Oxygen consumption analysis showed a small but significant increase in basal respiration of adipocytes derived from BAT SVF of Task1−/− compared to WT mice (Fig. 8A). UCP1-dependent respiration (oligomycin-uncoupled

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**Figure 5.** Morphologic features of Task1−/− WAT. Analysis of ia- (A–C) and scWAT (D–F) from WT and Task1−/− mice. A, D) WAT depot areas were evaluated in live animals by computed tomography of the same vertebral position (L5/L6). B, C, E, F) Hematoxylin and eosin-stained sections of WAT (B, E) were used to evaluate mean adipocyte size and distribution (C, F). Data are means ± sem (n = 8 mice/group). *P < 0.05. Scale bar, 100 μm.
respiration) and mitochondrial capacity (FCCP maximum mitochondrial respiration) were similar. In contrast, ADRB stimulation revealed a striking difference. Oxygen consumption of WT-derived adipocytes was strongly increased by addition of 100 nM isoproterenol (an ADRB pan-agonist), whereas this response was significantly lower in Task1−/−-derived adipocytes (Fig 8B). As expected, oligomycin did not reduce oxygen consumption in WT-derived adipocytes, in agreement with a total uncoupling of mitochondria due to UCP1 activity. The situation was different in Task1−/−-derived adipocytes where oligomycin still decreased β-adrenergic-activated mitochondrial respiration, demonstrating that mitochondria were not completely uncoupled and thus that UCP1 was not completely activated.

Then, we aimed to analyze whether the lower oxygen consumption was associated with a defect in β-adrenergic-induced lipolysis and gene expression. For this purpose, both glycerol release (Fig 9A) and Ucp1 mRNA levels (Fig. 9B) were measured in adipocytes derived from BAT SVF of WT or Task1−/− mice and treated with ADRB1, -2 and -3 agonists (dobutamine, terbutaline, and CL316243, respectively). Dobutamine and terbutaline responses were similar between WT- and Task1−/−-derived adipocytes. In contrast, a significant impairment of lipolytic activity and reduced Ucp1 mRNA levels in Task1−/−-derived adipocytes were observed after ADRB3 agonist treatment (Fig. 9A, B). This observation was confirmed at the protein level, as the UCP1 protein content in Task1−/− brown adipocytes stimulated with CL316243 was reduced compared with that in WT (Fig. 9C).

Adipocytes derived from scWAT SVF of Task1−/− and WT mice were similar in expression of adipogenic markers (Supplemental Fig. 2B, D, E), as well as in respiration capacity in the unstimulated condition (Supplemental Fig. 3A). However, adipocytes derived from Task1−/− mice
displayed a similar impaired β-adrenergic stimulation response except for lipolysis, which was not different between the 2 genotypes (Supplemental Fig. 3B, D). The impaired response to CL316243 stimulation may be related in part to the decrease in Adrb3 mRNA expression found in adipocytes derived from Task1^{2/2} mice (Supplemental Fig. 2B).

**Task1 knockout affects MR activation**

TASK1 has been reported to be involved in mineralocorticoid homeostasis (22, 36), and BAT has been described as an aldosterone target (37, 38). Hence, we investigated whether MR or glucocorticoid receptor (GR) activation is involved in the brown adipocyte dysregulation observed in Task1^{−/−} mice. Adipocytes derived from the BAT SVF of Task1^{2/2} and WT mice were treated with eplerenone (an MR-specific antagonist) or RU486 (mifepristone, a GR antagonist) and then stimulated with isoproterenol or CL316243 (Fig. 9D, E). Inhibition of the MR pathway by eplerenone restored ADRB3 stimulation, whereas inhibition of GR pathway by RU486 was inefficient (Fig. 9D). Conversely, WT-derived adipocytes treated with aldosterone, the natural ligand of MR, displayed an altered response to ADRB3 stimulation that was similar to that of Task1^{−/−}-derived adipocytes in the absence of aldosterone (Fig. 9E). Moreover, the aldosterone-induced decrease in Ucp1 expression was prevented by eplerenone pretreatment. Although, in addition to MR, aldosterone activates GR, our data did not support a role for GR, as in the presence of 1 μM aldosterone, the RU486 (a GR antagonist) did not restore the inhibitory effects, whereas eplerenone (a specific MR antagonist) antagonized the aldosterone efficiently. WT-derived adipocytes treated with A293 (a TASK1 blocker) impaired Ucp1 expression, which was restored by eplerenone, mimicking the Task1^{−/−} phenotype (Fig. 9E). The link between TASK1 and MR is confirmed by the fact that addition of aldosterone to Task1^{−/−} adipocytes did not induce an increased effect (Fig. 9D).

**DISCUSSION**

The potassium channel TASK1 represents a novel key component of BAT, as its expression was predominant in BAT, compared with scWAT and iaWAT, in the range of that observed in the heart, a tissue displaying a high TASK1 expression level (21). We demonstrated that Task1 expression in adipose tissue was confined to adipocytes and correlated with brown adipocyte activation in vivo, in agreement with recent reports (19, 29). Moreover, we characterized, for the first time, a decrease in Task1 expression in BAT and WAT of genetically obese mice. Our results are in line with the recently described association between the Kcnk3/Task1 locus and obesity in a large genome-wide association meta-analysis of body mass index (28).

Connection between TASK1 and obesity was corroborated by the knockout of the Task1 gene in mice, which led to a gain in white fat mass, particularly that caused by an...
increase in the size of intra-abdominal white adipocytes. This fat mass gain correlated with a decrease in BAT weight concomitant to a whitening of the tissue. In addition, Task1−/− mice BAT displayed a lower response to ADRB3 stimulation with decreased lipid substrate consumption. The decrease in BAT activity observed in vivo was reproduced in vitro in primary cultures. Indeed, Task1−/−-derived brown adipocytes displayed a reduced response to ADRB3 stimulation, with lower oxygen consumption, lower lipolysis, and reduced UCP1 expression. Thus, TASK1 is involved in key pathways of the thermogenic activity of brown adipocytes, and disruption of its function is associated with weight gain. Of note, adipocytes derived from BAT of Task1−/− mice displayed an unexpected slight increase in basal oxygen consumption in the unstimulated condition. This increase was not associated with substrate utilization and energy dissipation in vivo, as TG content was higher in Task1−/−

Figure 8. Functional analysis of brown adipocytes derived from BAT SVF. Differentiated brown adipocytes from BAT WT and Task1−/− mice were analyzed for oxygen consumption. Basal (A) and isoproterenol-stimulated (B) mitochondrial activity of WT- and Task1−/−-derived brown adipocytes was evaluated by measurement of OCR. The histograms correspond to mitochondrial respiration values. Data are means ± SEM of results of 3 experiments in 4 mice each. *P < 0.05.
brown adipocytes. We postulate that a defect in the mitochondrial capacity of Task1−/− adipocytes to use substrates accounts for this discrepancy. Further studies are needed to shed light on this phenomenon.

The BAT phenotype caused by inactivation of Task1 was similar to the phenotype obtained by MR stimulation in white and brown adipocytes [reviewed in Marzolla et al. (38)]. In vitro, aldosterone mimicked the Task1 knockout in WT adipocytes after ADRB3 activation. This response was restored by the aldosterone antagonist eplerenone. It has been reported recently that administration of an MR antagonist induces browning of WAT, with the appearance of

Figure 9. TASK1 deficiency affects the ADRB3 and MR pathways in brown adipocytes derived from BAT SVF. A, B) Lipolytic activity, assessed by glycerol release (A) and Ucp1 mRNA expression (B) of brown adipocytes, exposed to various ADRB agonist treatments [1 μM dobutamin (dobu), 1 μM terbutalin (terbu), or 1 μM CL316243] for 90 min (A) and 6 h (B). C) The UCP1 protein level evaluated by Western blot (normalized by β-tubulin band intensity) after a 6 h CL316243 treatment. D, E) Differentiated adipocytes from BAT SVF of Task1−/− (C) and WT (D) mice were treated for 1 d with various inhibitors or activators and then stimulated for the last 6 h with 1 μM isoproterenol or 1 μM CL316243. Eplerenone (Eple, 1 μM), RU486 (1 μM), and A293 (10 μM) were used as MR, GR, and TASK1 inhibitors, respectively. Aldosterone (100 nM and 1 μM) was used as an MR ligand. Ucp1 mRNA expression analysis was evaluated by RT-qPCR. Data are means ± SEM of data are from 3 experiments in 4 mice each. *P < 0.05.

TASK1 CONTROLS BROWN ADIPOCYTE ACTIVITY
brite/beige adipocytes (37). Herein, we show an increase in Task1 mRNA expression in the WAT of mice subjected to either ADRB stimulation or cold exposure, 2 situations associated with brite and beige adipocyte recruitment. This observation was confirmed in vitro with the SVF derived from scWAT and differentiated into white or brite adipocytes. In this context, we demonstrated the link between brite adipocyte differentiation and Task1 expression. Moreover, recruitment of brite adipocytes in scWAT was impaired in Task1–/– mice by ADRB3 agonist treatment. Taken together, these results show that TASK1 is a key protein of thermogenesis-competent adipocytes.

Altogether, these observations establish a link between Task1-knockout mice and MR activation; however, the precise mechanism remains to be elucidated. TASK1 channel activity is reported to control aldosterone secretion in adrenal glands (22), and Task1–/– female mice were shown to display severe hyperaldosteronism. In the present work, we used male mice that did not have high plasma aldosterone levels (22), and thus high levels do not explain the MR activation. Aldosterone is also known to be secreted by adipocytes (39). Analogous with the adrenal gland situation, one can assume that the absence of TASK1 in adipocytes results in hypersecretion of the hormone in a paracrine/autocrine manner. However, BAT Task1–/– and WT-derived adipocytes were both unable to secrete aldosterone under our culture conditions. Aldosterone was not detected in culture supernatants, and expression of Cyp11b2, the key enzyme of aldosterone synthesis, was found to be extremely low in white and brown adipocytes, the key enzyme of aldosterone synthesis, was found to be extremely low in white and brown adipocytes (40) and expression of Cyp11b2, the key enzyme of aldosterone synthesis, was found to be extremely low in white and brown adipocytes and was not increased in the absence of TASK1 (data not shown). As a potential mechanism for MR activation, one cannot rule out variation of MR activity through modulation of its phosphorylation state (40) and expression of undefined coactivators (41).

Even though aldosterone is a known ligand of GR (42), a putative involvement of GR in the Task1–/– phenotype can be excluded, given that RU486, a glucocorticoid antagonist, did not reverse either the TASK1-deficient BAT defect or the effect mimicked by aldosterone. However, glucocorticoids may represent the main MR ligand because MR displayed similar affinity for glucocorticoids and mineralocorticoids (43), and the plasma glucocorticoid level is up to 100-fold higher than that of aldosterone and increases in response to cold exposure or ADRB3 stimulation (44). It has been shown that GR activation by glucocorticoids inhibits Adrb3 and Ucp1 mRNA expression and UCP1 activity (45, 46). We have excluded GR involvement in the Task1–/– phenotype, but various mechanisms may explain MR activation by glucocorticoids. On the one hand, hydroxysteroid (11-β) dehydrogenase 2 (HSD11B2), an enzyme that inactivates cortisol through its oxidation into cortisone, has been shown to contribute to a protective mechanism for MR activation in epithelial tissue (47). On the other hand, HSD11B1, which transforms cortisone into active cortisol, contributes to increase MR activation. Neither Hsd11b1 nor Hsd11b2 mRNA expression was affected in TASK1-knockout–derived adipocytes (data not shown). These observations therefore do not support the involvement of these enzymes in the control of MR response.

In conclusion, we demonstrated a key function of TASK1 in the control of the β-adrenergic response of brown and brite adipocytes. In conditions where the adrenergic response to cold is accompanied by an increase in circulating glucocorticoid levels, it is tempting to postulate that TASK1 preserves brown adipocytes against the deleterious effect of glucocorticoids during thermogenesis by controlling MR activity. TASK1 represents a master effect of general metabolism, as it is now found to control key metabolic functions, including plasticity of WAT and the thermogenic function of brown adipocytes (present work), aldosterone secretion by the adrenal gland, and insulin secretion by β-pancreatic cells (22, 48). This novel function of TASK1 emphasizes recent published results in humans and raises the possibility that TASK1 also plays an important role in human adipose function, in relation to energy balance and metabolic diseases (29) (28).

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