BMP4 and BMP7 induce the white-to-brown transition of primary human adipose stem cells

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Adipose tissue (AT) plays a crucial role in the regulation of energy homeostasis. Functionally, AT can be subdivided into white AT and brown AT. While white AT is the main site of energy storage and provides substrates in terms of energy needs by releasing free fatty acids and glycerol, brown AT metabolizes triglycerides to generate heat in adaptation to a cold environment (32). This unique function of brown AT is due to a high mitochondrial density and to the presence of uncoupling protein 1 (UCP1). Until recently, brown AT was believed to play a negligible role in the adult human. However, it gained substantial interest since active brown AT has been shown to be present in adults by five independent groups (4, 23, 38, 43) and brown AT activity was negatively associated with increasing body mass index (BMI) (23, 38, 43). Furthermore, UCP1-expressing brown-like adipocytes have been discovered within white AT after cold exposure (31). These so called “brite” (brown-in-white) adipocytes arise from white preadipocytes (19), whereas classical brown adipocytes are derived from the myogenic lineage (29, 35). Both increasing brown AT activity and promoting the induction of brite adipocytes in white AT represent strategies to counteract obesity. Pharmacological agents, like peroxisome proliferator-activated receptor-γ (PPARγ) agonists (8, 20), triiodothyronine (T3) (12), cardiac natriuretic peptides (CNP) (1), and bone morphogenetic protein (BMP) 7 (27) have been reported to induce brite adipocytes in humans.

BMPs belong to the transforming growth factor-β (TGF-β) superfamily and are important regulators of developmental processes. BMP signaling is complex and dependent on the receptor composition of a cell and the presence of intra- and extracellular antagonists (3). Selected members of the BMP family play an important role in the regulation of white vs. brown adipogenesis and energy homeostasis (16). While BMP4 induces commitment of pluripotent stem cells to the white adipocyte lineage (2, 10) and promotes differentiation of brown adipocyte lineage determination of the multipotent C3H10T1/2 cell line and promotes differentiation of murine brown preadipocytes (37). Evidence from transgenic mouse models suggests that BMP7 plays an important role in whole energy homeostasis, since BMP7 knockout mice displayed a decrease in brown AT mass (37) and systemic BMP7 administration to ob/ob mice led to increased energy expenditure and reduced food intake accompanied by weight loss (36). Therefore, it is currently assumed that BMP4 and BMP7 differentially regulate white vs. brown adipogenesis. However, BMP4 might also induce a white-to-brown switch and have positive effects on energy homeostasis (21). Qian et al. (21) showed that AT-specific overexpression of BMP4 leads to induction of

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brite adipocytes and increased energy expenditure in mice. Furthermore, murine 3T3-L1 adipocytes challenged with BMP4 during differentiation displayed a brite phenotype with increased mitochondrial content and expression of brown AT markers (21).

There is evidence that the molecular signature from human AT depots differ from mouse AT depots (42), raising the question if results from mouse studies investigating mechanisms of browning of white AT can be extrapolated to humans. Furthermore, the effects of different BMPs on white vs. brown adipogenesis seem to be dependent on the AT depot and species studied. The impact of BMP4 and BMP7 on browning of white adipocytes has not yet been explored in parallel in a human cell culture model. Therefore, we aimed to investigate for the first time the effect of BMP4 on the white-to-brown transition of primary human adipose stem cells (hASCs) compared with the known browning factor BMP7. In this study, hASCs were isolated from the subcutaneous depot of different donors and challenged with BMP4 or BMP7. In contrast to mice, in humans both BMP4 and BMP7 were found to induce a brite adipocyte phenotype, suggesting substantial differences in AT plasticity between different species.

MATERIALS AND METHODS

Isolation and culture of primary hASCs. Subcutaneous AT (from the abdominal and mammary region) was obtained from healthy lean or overweight women (n = 38; BMI: 28.8 ± 0.7 kg/m²; age: 39 ± 3 yr) undergoing plastic surgery. The procedure was approved by the ethical board of Heinrich-Heine-Universität, Düsseldorf, Germany. hASCs were isolated by collagenase digestion of AT as previously described by our group (6). Isolated cell pellets were resuspended in adipocyte basal medium (BM), which was DMEM/F-12 (GIBCO, Grand Island, NY) supplemented with 14 nmol/l NaHCO₃, 33 mmol/l biotin, and 17 mmol/l α-panthenoic-acid containing 10% FCS (GIBCO) at pH 7.4; seeded in six-well plates; and maintained at 37°C and 5% CO₂. When cells reached confluence, differentiation was induced with adipocyte differentiation medium (BM supplemented with 66 nM insulin, 1 nM triiodo-l-thyronine, 100 nM cortisol, 10 mg/ml apo-transferrin, and 50 mg/ml gentamycin) with the addition of 50 ng/ml BMP4 or BMP7. After 14 days, cells were differentiated for 14 days or as indicated. Recombinant human BMP4 or BMP7 (R&D Systems, Minneapolis, MN) was added to the differentiation medium at a final concentration of 50 ng/ml.

Oil Red O staining. For Oil Red O Staining, cells were cultured in six-well plates and differentiated with or without BMP4 or BMP7. After 14 days, cells were washed in PBS and fixed for 2 h with a solution containing 71% picric acid (vol/vol), 24% acetic acid (vol/vol), and 5% formaldehyde (wt/vol). Afterwards, cells were washed three times with PBS and lipids were subsequently stained with 0.3% Oil Red O dissolved in 60% isopropanol for 10 min. The staining was quantified by dissolving Oil Red O with 100% isopropanol and measuring absorbance at 500 nm.

Immunofluorescence staining. For immunocytochemistry, hASCs were cultured on 12 × 12 mm coverslips coated with 0.1% gelatine. After 12 days of differentiation, adipocytes were washed with PBS and fixed with 3% formaldehyde for 15 min at room temperature. Afterwards, cells were washed three times with PBS for 5 min and incubated with blocking solution (PBS containing 1% BSA and 0.1% Triton X-100) for 1 h at room temperature. Mouse anti-human MTCO2 (Abcam, Cambridge, UK) was diluted 1:100 in blocking solution and incubated at 4°C overnight. After three washes for 5 min with PBS, cells were incubated with anti-mouse Alexa Fluor 488 (Life Technologies, Carlsbad, CA) diluted 1:500 in blocking solution for 1 h at room temperature. Nuclei were stained with 1 μg/ml DAPI (Life Technologies) in PBS for 10 min at room temperature. Finally, coverslips were mounted with ProLong Gold Antifade reagent (Life Technologies). Cells were analyzed using a Zeiss fluorescence microscope (Oberkochen, Germany) equipped with a Axio Cam MRc5. Images were acquired with AxiosVision rel. 4.3 with equal settings for contrast/brightness and merged by use of the ImageJ software.

Generation of concentrated supernatants. For generation of supernatants, hASCs were seeded in 75-cm² flasks and underwent adipogenic differentiation for 14 days. Subsequently, cells were incubated with 10 ml of αMEM (GIBCO) for 24 h. Afterwards, supernatants were collected, centrifuged at 1,200 rpm, and concentrated 100-fold by using Amicon Ultra-4 Centrifugal Filter Units with Ultraclacc 3 membrane (Millipore, Billerica, MA). Concentrated supernatants were diluted in Laemmli buffer and subjected to immunoblot analysis.

Immunoblot analysis. Total cellular proteins were dissolved in lysis buffer containing 20 mM MOPS, 2 mM EGTA, 5 mM EDTA, 1 mM dithiothreitol, 1% Triton X-100, complete protease inhibitor cocktail (Roche, Basel, Switzerland), and PhosStop phosphatase inhibitor cocktail (Roche) at pH 7.0. Lysates were shaken for 2 h at 4°C and centrifuged at 10,000 g for 20 min. Protein concentration was determined by using Bradford protein assay (Bio-Rad, Hercules, CA), and 10 μg protein were separated by SDS-PAGE using 10% horizontal gels. Proteins were transferred to a polyvinylidene difluoride membrane (Millipore) in a semidry blotting system. Membranes were blocked with 5% nonfat dry milk or BSA in TBS containing 0.1% Tween for 1 h at room temperature and probed with the indicated primary antibodies overnight at 4°C. After being washed, membranes were incubated with a secondary horseradish peroxidase (HRP)-coupled antibody and processed for enhanced chemiluminescence detection using Immobilon HRP substrate (Millipore). Signals were visualized and analyzed on a Bio-Rad VersaDoc 4000 MP work station. Antibodies were used as follows: anti-BMP4 (ab93939; Abcam) diluted 1:1,000, anti-BMP7 (sc-9305; Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:200, anti-phospho SMAD1/5/8 (Abcam, Cambridge, UK), anti-MTCO2 (Abcam, Cambridge, UK) were diluted 1:1,000, anti-OXPHOS cocktail (MS604; Abcam) diluted 1:1,000, and anti-beta actin (ab6267; Abcam).

High-resolution respirometry. For oxygen consumption measurements, hASCs were seeded in T25-cm² flasks and differentiated for 14 days under control conditions or with the addition of 50 ng/ml BMP4 or BMP7 (R&D Systems, Minneapolis, MN) was added to the differentiation medium at a final concentration of 50 ng/ml.

1 This article is the topic of an Editorial Focus by Maria-Jesus Obregon (17a).
BMP4 AND BMP7 PROMOTE BROWNING OF hASCs

UCP1 (Hs_UCP1_3_SG), TCF21 (Hs_TCF21_2_SG), ZIC1 (Hs_ZIC1_1_SG), PPARγ coactivator 1 (PGC-1α; Hs_PPARC1A_1_SG), and β-actin (Hs_ACTB_2_SG) and primer pairs for CD137 (Eurofins MWG, Hamburg, Germany; forward 5′-AGCTGTTACAACATAGTAGCCAC and reverse 5′-TCTCTGCAATGACTTGTCCTCT) on a Step One Plus cycler (Applied Biosystems, Carlsbad, CA). Amplification was done as follows: one step at 95°C 2 min, 40 cycles at 95°C 15 s, 55–58°C 30 s, and 60°C 30 s. All samples were analyzed in triplicate. Expression of genes was normalized to β-actin mRNA according to the comparative threshold method (ΔCt).

Microfluidic card TaqMan gene expression assay. RNA integrity was tested on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) using Agilent RNA Nano chips. Only RNAs with a RIN score of 7.5 or higher were used for analysis. Synthesis of cDNA was done from 0.5 μg of each total RNA preparation in a volume of 20 μl with the QuantiTect Reverse Transcription Kit (Qiagen) according to the manufacturer’s instructions. Thermal cycling of the PCR reactions was done in microfluidic cards on a Viia7 Real Time PCR 384 well cycler and fluorescence plate reader (Applied Biosystems). Specific TaqMan Gene Expression Assays (Applied Biosystems) for PRDM16 (Hs00991677_m1), PPARα (Hs00947539_m1), PGC-1β (Hs00991677_m1), NRF1 (Hs0192316_m1), TFAM (Hs00273372_s1), OXR1 (Hs00250562_m1), UCP2 (Hs01075227_m1), UCP3 (Hs01106052_m1), CYCS (Hs01588974_g1), VDAC1 (Hs01631624_gH), GAPDH (Hs02758991_g1), and β-actin (Hs01060665_g1) were used. Expression levels of genes were normalized to GAPDH and β-actin as housekeeping genes and related to control samples.

Statistics. Results are expressed as means ± SE. One-way ANOVA (post hoc test: Bonferroni or Dunnett) was used to determine statistical significances. All statistical analyses were done using GraphPad Prism 5 considering P < 0.05 as statistically significant.

RESULTS

BMP4 is secreted by human adipocytes. BMP4 has been shown to induce commitment of pluripotent C3H10T1 cells to the adipocyte lineage (2, 33). Furthermore, BMP4 mRNA is induced in primary human preadipocytes undergoing adipogenic differentiation (9), suggesting that BMP4 may be a secreted factor acting on adipocyte differentiation in a paracrine manner. Thus we first examined BMP4 protein levels in primary hASCs undergoing adipogenic differentiation. BMP4 is constitutively expressed during differentiation of primary hASCs, with a trend towards a decrease at later stages of differentiation (Fig. 1A). Moreover, we show for the first time that BMP4 is secreted from differentiated hASCs, as determined by immunoblot of concentrated supernatants from seven different donors (Fig. 1C). The detected band for BMP4 has a molecular mass of 47 kDa, corresponding to the uncleaved propeptide of BMP4 (UniProt entry P12644). We also assessed BMP7 protein levels during adipogenesis of hASCs. We found that BMP7 (UniProt entry P18075) is present as a 50-kDa form, representing the precursor (data not shown), and at a smaller 15.7-kDa protein, reflecting the cleaved mature protein (Fig. 1B). Levels of mature BMP7 were slightly but not significantly upregulated during adipogenesis (Fig. 1B). Interestingly, we could not show secretion of BMP7 from differentiated hASCs, since BMP7 was not detectable in the same concentrated supernatants used for detection of BMP4 (Fig. 1D).

To compare the effect of BMP4 to BMP7 on the white-to-brown shift of hASCs, we determined equipotent concentrations of BMP4 and BMP7. Therefore, undifferentiated hASCs were challenged with different concentrations of BMP4 or BMP7 for 30 min and SMAD1/5/8 phosphorylation, the main signaling pathway activated by BMPs, was measured. We observed a dose-dependent increase of BMP4- and BMP7-induced SMAD1/5/8 phosphorylation with similar maximal concentrations of 50 ng/ml for BMP4 and BMP7, which induced independent (Fig. 2B). For long-term treatments we chose a concentration of 50 ng/ml for BMP4 and BMP7, which induced

![Fig. 1. Bone morphogenetic protein 4 (BMP4) but not BMP7 is secreted from differentiated hASCs. A and B: human adipose stem cells (hASCs) were differentiated for the indicated time points and BMP4 (A) and BMP7 (B) protein levels were analyzed by immunoblot. All data are normalized to β-actin and expressed relative to day 0. Data are expressed as mean values ± SE; n = 5. C and D: to analyze secretion of BMP4 and BMP7 from adipocytes, supernatants of hASCs from 7 different donors at day 14 of differentiation were collected after 24 h, 100× concentrated, and subsequently analyzed by Western blot for BMP4 (C) and BMP7 (D). F9: F9 cell lysate (sc-2245; Santa Cruz Biotechnology) was used as a positive control for BMP7; ID: immunodetection.](http://ajpcell.physiology.org/content/early/2010/03/30/ajpcell.00290.2013/F001106052_m1)
maximal SMAD1/5/8 phosphorylation. Previous studies in preadipocytes and mesenchymal stem cells used similar BMP concentrations (9, 10, 15, 21, 27, 37).

**BMP4 and BMP7 induce a brite-like expression profile in hASCs.** It is known that BMPs play a role in preadipocyte commitment and also promote differentiation of already committed preadipocytes (28). Therefore, we first investigated the effect of chronic BMP4/7 exposure on hASC differentiation. Differentiation efficiency of hASCs was significantly increased by long-term BMP4 and BMP7 treatment, as indicated by Oil Red O Staining (Fig. 2C) and PPARγ expression (Fig. 3A).

Moreover, BMP4 and BMP7 induced a brown-like mRNA expression profile in hASCs, as determined by significantly increased UCP1 expression (Fig. 3B) and reduced expression of the white-specific marker TCF21 (Fig. 3C). ZIC1, a marker for classical brown adipocytes derived from the myogenic lineage, was not altered (Fig. 3D). The transcription factor PRDM16 is a key regulator of brown adipogenesis and activates the PPARγ coactivator 1 (PGC-1) family members PGC-1α and PGC-1β (30), which play a role in the regulation of mitochondrial function (24). The key regulator PRDM16 was slightly, but not significantly, upregulated by BMP4 and BMP7 (Fig. 3F). While PGC-1α expression was also not enhanced by BMP4 and BMP7 (Fig. 3E), PGC-1β expression was significantly increased by BMP4 and BMP7 (Fig. 3F). Since mitochondrial content and function is enhanced in brite adipocytes (19), we studied mRNA expression levels of further genes encoding for transcriptional regulators of mitochondrial function and mitochondrial proteins. The transcription factors NRF1 and TFAM, which are involved in mitochondrial biogenesis (25), were not affected by BMP4 or BMP7. Expression of some genes like CYCS and UCP2 was significantly enhanced by BMP4 and BMP7, whereas expression of OXR1 and UCP3 was downregulated in BMP4- and BMP7-treated hASCs (Fig. 3F).

The ability to induce UCP1 in response to BMP4 and BMP7 was highly variable between donors (1.4- to 13.9-fold and 1.5- to 17.2-fold, respectively; Fig. 4A). Recently, CD137 and TMEM26 have been described as brite adipocyte-selective markers and murine precursor cells expressing high levels of CD137 showed a higher potential to induce browning in response to the newly identified myokine irisin (42). Therefore, we measured CD137 mRNA levels in undifferentiated hASCs of nine donors and separated them into CD137 low- and high-expressing group (Fig. 4B). Interestingly, the mean BMI was similar in the CD137 low- and high-expressing group (29.3 ± 1.3 and 28.9 ± 1.2 kg/m², respectively). CD137 high-expressing donors showed a significantly higher induction of UCP1 in response to BMP4 and BMP7 than CD137 low-expressing donors (Fig. 4D), while there is no significant difference in PPARγ expression between the two groups (Fig. 4C).

**Mitochondrial content and function are not increased in hASCs by BMP4 and BMP7.** Next, we aimed to assess if the observed browning effects of BMP4 and BMP7 on UCP1 and mitochondrial gene expression resulted in a higher mitochon-
BMP4 and BMP7 promote browning of hASCs

A large number of adipokines act on AT in an auto-/paracrine manner and exert endocrine effects (7, 34). Here we show that BMP4 is a novel adipokine secreted from hASCs. BMP4 is expressed and secreted from proliferating A33 cells, which is an adipogenic subline of the pluripotent 10T1/2 cell line (2). Furthermore, BMP4 mRNA is induced in human preadipocytes undergoing differentiation. The inhibition of adipogenic differentiation by the BMP4 inhibitor noggin strongly suggests a paracrine function for BMP4 in adipogenesis (9). Our results confirm that BMP4 is secreted from differentiated hASC and promotes adipogenic differentiation of hASCs. It should be noted that we observed secretion of a 47-kDa BMP4 peptide, corresponding to the uncleaved propeptide of BMP4. BMPs are described to be fully processed to their mature form before secretion (26). However, BMP4 and other BMP members can form complexes with their prodomains after cleavage and be secreted in this form (3). Since we were not able to show BMP7 secretion, BMP7 does not probably act on adipogenesis in an auto-/paracrine manner. In line with our findings, BMP4 and BMP7 increased lipid accumulation in murine brown preadipocytes (37) and BMP7 promoted adipogenesis of human preadipocytes derived from white AT (27).

Besides the effect of BMP4 and BMP7 on adipogenic differentiation of hASCs, we observed gene expression pat-
The ability of different donors to induce UCP1 expression in response to BMP4/7 is variable between donors and related to CD137 expression. A: PPARγ and UCP1 expression in hASCs at day 14 of differentiation in response to BMP4 and BMP7 was assessed by quantitative RT-PCR. Some donors show a very strong induction of UCP1 in response to BMP4/7, while the effect on PPARγ is comparable between donors. Data are normalized to β-actin and expressed related to control; n = 16. B: undifferentiated hASCs from 9 different donors were analyzed for CD137 expression by quantitative RT-PCR and separated into CD137 high (n = 3)- and low (n = 6)-expressing donors. There was no significant difference in body mass index (BMI) between the CD137 low and CD137 high group (29.3 ± 1.3 and 28.9 ± 1.2 kg/m², respectively). C and D: comparison of PPARγ (C) and UCP1 (D) mRNA expression levels between CD137 low- and high-expressing donors on day 14 of differentiation. Donors expressing high levels of CD137 show a significant stronger induction of UCP1 in response to BMP4 and BMP7 compared with CD137 low expressing donors. Data are mean values ± SE. *p < 0.05 CD137 low vs. CD137 high.

Fig. 4. The ability to induce UCP1 in response to BMP4/7 is variable between donors and related to CD137 expression. A: PPARγ and UCP1 expression in hASCs at day 14 of differentiation in response to BMP4 and BMP7 was assessed by quantitative RT-PCR. Some donors show a very strong induction of UCP1 in response to BMP4/7, while the effect on PPARγ is comparable between donors. Date are normalized to β-actin and expressed related to control; n = 16. B: undifferentiated hASCs from 9 different donors were analyzed for CD137 expression by quantitative RT-PCR and separated into CD137 high (n = 3)- and low (n = 6)-expressing donors. There was no significant difference in body mass index (BMI) between the CD137 low and CD137 high group (29.3 ± 1.3 and 28.9 ± 1.2 kg/m², respectively). C and D: comparison of PPARγ (C) and UCP1 (D) mRNA expression levels between CD137 low- and high-expressing donors on day 14 of differentiation. Donors expressing high levels of CD137 show a significant stronger induction of UCP1 in response to BMP4 and BMP7 compared with CD137 low expressing donors. Data are mean values ± SE. *p < 0.05 CD137 low vs. CD137 high.

An interesting observation of this study was the variable response to BMP4/7 in those donors highly expressing CD137. Thus CD137 might also be a marker for brite precursor cells in humans. However, the comparability between mouse and human AT depots is currently puzzling. Wu et al. (42) suggested, that brown AT from the adult human consists of brite adipocytes rather than of classical brown adipocytes derived from a myogenic origin. However, recent studies provided evidence for the existence of classical brown AT in humans (5, 11, 13), indicating that human brown AT is composed of brite and classical brown adipocytes. It should be noted that active brown AT cannot be found in all humans and decreases with increasing BMI (23, 38, 43). Vice versa, brown AT can be recruited in humans and morbidly obese patients negative for brown AT became brown AT-positive after weight loss. Interestingly, not all patients were able to recruit brown AT (39). In line with that, our results indicate that not all individuals might have the ability to induce thermogenically active adipocytes. To counteract human obesity by targeting brite/brown AT, it is of importance to identify the developmental origins of these tissues in humans and to identify markers that label cells capable to induce thermogenically active adipocytes.

Assessment of white-to-brown transition solely based on UCP1 expression without any functional readout is critical,
since expression of UCP1 mRNA does not necessarily lead to increased energy dissipation (17). To strengthen this study we analyzed mitochondrial biogenesis and function, which are enhanced in brite adipocytes. Increased mitochondrial biogenesis can be induced in human adipocytes by certain stimuli, such as CNPs (1), T3 (12), and PPARγ agonists (8, 20). Here, we found that BMP4 and BMP7 upregulate OXPHOS complexes in hASCs. This effect probably reflects the increased differentiation induced by BMP4 and BMP7, since mitochondrial content is upregulated during differentiation of 3T3-L1 adipocytes (41), rat adipocytes (14), and also in our human cell model. Immunofluorescence staining for MCT02 confirmed that there is no difference in MCT02 abundance between the differentiated lipid-loaden hASCs treated with and without BMP4/7. The increased oxygen consumption in BMP4/7 challenged hASCs can therefore also be related to the differentiation-induced increased expression of OXPHOS complexes.

Fig. 5. Mitochondrial content and function in differentiated hASCs is not increased by BMP4 and BMP7. hASCs from different donors were induced for adipogenic differentiation under standard conditions or with the addition of recombinant human BMP4 or BMP7 (50 ng/ml). Medium and recombinant BMP4 and BMP7 were replaced every 2–3 days. A: on day 14 of differentiation cells were lysed and analyzed for OXPHOS complexes by immunoblot with a commercially available OXPHOS antibody cocktail. BMP4 and BMP7 increased protein levels of the single complexes and of OXPHOS complexes in total. Results are normalized to β-actin protein levels and expressed relative to control. Data are mean values ± SE; n = 9. ***P < 0.001, **P < 0.01, *P < 0.05. Representative blots are shown. B: OXPHOS complexes in differentiated hASCs (day 14) compared with undifferentiated hASCs (day 0). Results are normalized to β-actin and expressed relative to day 0. Data are mean values ± SE; n = 5–6. *P < 0.05. Representative blots are shown. C: basal and uncoupled respiration was measured in differentiated hASC at day 14. Data are mean values ± SE; n = 4. *P < 0.05 vs. control. D: immunofluorescence staining against mitochondrial marker MTC02 (green) and staining of nuclei with DAPI (blue) in hASCs differentiated under control conditions and challenged with BMP4 or BMP7. MTC02 abundance is higher in differentiated hASCs (opened arrowhead) compared with nondifferentiated hASCs (closed arrowhead).
tion effect of the BMPs. Mitochondrial biogenesis in AT is mainly controlled by members of the PGC-1 family (24). Of note, PGC-1β, but not PGC-1α, was upregulated in differentiated hASCs by BMP4/7 treatment. In line with that, PGC-1β has been proposed to be involved in general mitochondrial biogenesis in white AT, while PGC-1α plays a role in brown AT mitochondrial function (18). Nevertheless, BMP7 is able to enhance mitochondrial biogenesis in murine brown preadipocytes accompanied by increased PGC-1α expression (37) and overexpression of BMP4 in murine white AT increased PGC-1α mRNA levels and mitochondriogenesis (21). Thus BMP4 and BMP7 can affect mitochondrial biogenesis in murine models but were not able to increase brown-related mitochondrial biogenesis in our human cell model. To our knowledge, there are no reports on the role of BMP4 and BMP7 on mitochondria in primary human adipocytes. Taken together, we showed here that BMP4 and BMP7 induce a brite gene expression pattern in hASCs but do not induce a full white-to-brown transition with increased mitochondrial function. AT plasticity is a complex scenario, and other AT-derived factors may be necessary to synergize with BMP4 and BMP7 to induce complete browning in human white AT.

Our findings differ from the current view of BMP4 as a player in white adipogenesis and BMP7 as promoter of brown adipogenesis (16, 28, 37). Importantly, this view is based on different studies using different cell model systems (murine vs. human; brown AT vs. white AT) and the effects of BMP4 and BMP7 have been compared only in a few cases. In the study by Tseng et al. (37), BMP4 and BMP7 had the same effect on lipid accumulation in murine brown preadipocytes, but only BMP7 was able to significantly induce UCP1 expression whereas BMP4 suppressed expression of UCP1 in these brown preadipocytes. The induction of UCP1 mRNA expression by BMP7 has also been shown in primary human preadipocytes derived from subcutaneous AT. But a longer treatment with BMP7 was necessary to induce browning in human preadipocytes than in murine preadipocytes and the effect on UCP1 expression was less strong (27). Furthermore, only a slight effect of BMP7 on UCP1 expression has been observed in human multipotent adipose-derived stem cells (20). We also show a slight effect of BMP7 on UCP1 expression and no enhancement of mitochondrial function, suggesting a lower potency of BMP7 to induce browning in human preadipocytes compared with murine cells. Moreover, BMP4 did not suppress UCP1 expression in hASCs but exerted similar effects as BMP7 on all aspects of white-to-brown transition in primary hASCs. The inhibitory effect of BMP4 on brown adipogenesis, as shown in murine brown preadipocytes (37), has never been investigated in human white preadipocytes. Furthermore, there was recent evidence that BMP4 plays a different role in white and brown...
AT. In transgenic mice with AT-specific overexpression of BMP4, mRNA levels of UCP1 and the key factors of brown adipogenesis PGC-1α and PRDM16 were strongly increased in white AT, while the expression of these genes were decreased in brown AT (21). The myokine irisin is another example uncovering differences between mice and humans. While irisin induced browning in murine preadipocytes derived from white AT (42), we recently showed that irisin has no effect on white-to-brown transition in human white preadipocytes (22). Comparing our results with these previous studies supports the idea that there are discrepancies of the action of BMPs between human vs. murine models and white vs. brown AT precursor cell origin (summarized in Fig. 6). Additionally, AT plasticity is a complex scenario and rather a cocktail of factors than BMP4 and BMP7 alone may be necessary to induce a complete white-to-brown transition in human white AT.

Given this and taking into account, that BMP signaling is dependent on the receptor composition, the presence of ligands, and the extracellular antagonists (3), it is likely that BMP4 and BMP7 might have a similar effect on precursor cells derived from white AT but play a differential role in brown precursor cells. In addition, BMP4 and BMP7 may be more potent inducers of browning in mice than in humans. Furthermore, we here show that BMP4 is a new adipokine and acts on adipogenesis and white-to-brown transition in an auto-/paracrine manner. It has been shown recently that BMP4 expression is higher in white AT from lean compared with obese humans (21). Therefore, BMP4 represents a potential target in this and taking into account, that BMP signaling is dependent on the receptor composition, the presence of ligands, and the extracellular antagonists (3), it is likely that BMP4 and BMP7 might have a similar effect on precursor cells derived from white AT but play a differential role in brown precursor cells. In addition, BMP4 and BMP7 may be more potent inducers of browning in mice than in humans. Furthermore, we here show that BMP4 is a new adipokine and acts on adipogenesis and white-to-brown transition in an auto-/paracrine manner. It has been shown recently that BMP4 expression is higher in white AT from lean compared with obese humans (21). Therefore, BMP4 represents a potential target in human white AT.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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